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FOREWORD

This technical session was sponsored jointly by the Cane Sugar Refining Research Project, Inc., and the Southern Regional Research Center, Agricultural Research Service, U. S. Department of Agriculture. The program was assembled by Dr. F. G. Carpenter and Dr. M. A. Clarke; the local arrangements Chairman was George W. Muller, Jr.; the Conference Coordinator was Shirley T. Saucier, and these Proceedings were edited by Dr. Margaret A. Clarke.

The technical sessions are held every other year to provide for an exchange of information among technical leaders in the cane sugar industry, and to report on research and recent developments of benefit to the cane sugar refining industry.

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DEITZ AND CARPENTER REVIEWED

By Kenneth R. Hanson[1]

ABSTRACT

Between January 1946 and July 1963 the Bone Char Research Project Inc. issued 76 reports of its work. This body of literature contains the results of one of the most intensive and best coordinated research efforts into sugar ever undertaken. While some of the research, particularly in the analytical field, has found everyday use, many of the bone char investigations have gone unused. This paper reviews the highlights of these reports and points out studies where further development may lead to improvements in refinery technology.

INTRODUCTION

The story, albeit a somewhat apocryphal version, of the events leading to the initiation of the Bone Char Research Project has been related often enough elsewhere that it is not necessary to retell it here. On a personal note, I might say that as an employee of the McCahan Refinery in the late 1930's, I was kept informed of the early progress of the Project by Paul Woehrle, and had the pleasure of taking Dr. Victor R. Deitz through the McCahan char house. This was one of his first, if not the very first, such inspection trips. One outcome of these char house visits was the publication in 1947 of the report, "Preliminary Survey of Bone Char Revivification and Filtration" by Victor R. Deitz (13)[2]. The foreword to this report states that the study was instigated for a dual purpose:

1. To make original observations in the refinery in order to correlate the previous laboratory findings from fundamental researches with the various phases of the revivification process.

2. To familiarize the Bureau laboratory staff with the commercial operations of the char house of a sugar refinery.

It is a commentary on the rate of advancement of the art of char house operation that this report remains one of the most practical reference volumes on char house practice available today.

The initial researches of the Project, referred to above, considered the fundamentals of bone char as an adsorbent, and had two specific goals:

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[2]Numbers in parentheses refer to items under "References" at the end of this paper.

1. To determine the effects due predominantly to variations in the amount of available surface area.

2. To determine the effects due to specific chemical reactivity.

PROJECT ACTIVITIES

Publications and Technical Sessions

The major monuments of the project consist of the 76 quarterly reports covering the period from January, 1946 to June, 1963. These trace a growing sophistication in the progress of bone char chemistry. The first reports deal with traditional sugar house analysis in terms of color, Brix, invert and pH; the later reports cover complex investigations using advanced analytical equipment. Beside them, in my bookcase, are the bound volumes containing the papers presented at the seven Technical Sessions on Bone Char held between 1949 and 1961. At these Sessions was developed the type of program that we are following today at this Session of the Cane Sugar Refining Research Project: contributions by technologists of member companies, and progress reports from the scientists of the Project, with extensive discussion of each paper.

Analytical Developments

Many individual investigations were conducted by the Bone Char Project and eventually it became necessary to develop analytical techniques that would generate accurate and, perhaps even more important, pertinent data. Initially most investigations concerned bone char itself and its chemical and physical properties; from these studies an understanding was built as to how and why bone char works. It was early confirmed that bone char in the refining process has several functions: color removal, ash removal and buffer capacity. Since bone char then cost 10¢/lb it was important to conserve this expensive refining aid. Research emphasis was therefore placed on operating and regeneration conditions. Some of the highlights of investigations conducted during the life of the Project will be reviewed.

Historical Investigation

Most projects of this type delve at some point into industrial history, and with this Project the history of American char practice was traced in Report 27 (15) using the char house of Mathiessen and Wiecher's Jersey City Refinery as an example. The original filters were 3 ft in diameter by 30 ft high with flat bottoms. After washing, char was washed into "sour tubs" and the adsorbed organics allowed to ferment or "sour". In the virtual absence of conveying equipment the "soured" char was spread out on the floor above the kilns to dry partially, and then was shoveled by hand into the retorts.

At that time char was regenerated by two cheap agents: water and fuel. Today it still regenerated with the same two agents, although these are no longer cheap. Most of the advances in char house practice between 1873 and 1890 were in materials handling equipment rather than in process variables. Liquor before char filtration was defecated with black paste (a mixture of char dust and hydrochloric acid) containing 12% to 14% available monocalcium phosphate, and filtered through bag filters. The result, when defecation was properly conducted, was a highly polished clean liquor. There is even a trend today to return to this old type of pnosphate defecation, which was abandoned when centrifugal pumps were introduced because they destroyed the phosphate floc. Raw sugar was about 93° polarization at that time, and the char requirement was 4 lb char to 3 lb raw sugar, with 64% of the char on affination sirup (all put over char in those days) and 36% on washed raw sugar. Our progress since 1890 has been in degree, not in kind.

Surface Area Investigation

Many of the investigations made by the Project are still pertinent to the efficient operation of a sugar refinery using bone char as a basic refining aid. The report for the last quarter of 1954, later published in article form (36), summarized information about the surface area of bone char. The data show that new char has a surface area of about 120 m^2/g whereas discard char is down to about 30 m^2/g. Although it had been long known that char loses porosity in use, these quantative data showed for the first time the extent of this loss. The research was done using B.E.T. apparatus for surface area determination with liquid nitrogen, a technique that is beyond the financial and technical means of the average sugar refinery. Recently Bennett and Abram of Tate and Lyle published their techniques for determination of surface areas, both total and of carbon only, with solutions of surface active agents (1). Using these techniques, any refinery with a careful chemist can use surface area measurements to evaluate its char and to help control char condition.

Char Washing

Other research was more pragmatic, as, for example, the report for the third quarter of 1948 (14) which discussed the optimum carbon content of bone char and the rate of washing char. Introduced in that report were some heretical comments on the possibility of washing char with water of a temperature less than 200°F, since some mineral salts, calcium sulfate in particular, are more soluble in cold than hot water. To justify this reduction in temperature, the potential saving of energy was mentioned as oil was selling at the then outrageous price of 7.6¢/gal. Of course in those days, when it was common practice to press and char filter all affination sirup, char usage was much greater than it is now, with an average of 100 lb of char burned per 100 lb of melt.

Char Kilning

Several reports were written on the other phase of char regeneration, kilning, the most definitive being Report 60 by Dr. Carpenter in January 1961

(2, 3). Again the analytical theme was repeated: whereas early research had used the conventional measuring tools of pH of water extract, removal of "color" and "ash," and lye tests, studies soon disclosed that controlled experiments using advanced analytical techniques were necessary. A temperature of 500°C in a low oxygen or inert atmosphere was found to give char with the best color removal characteristics, although the ability to remove calcium and magnesium still would increase with regeneration temperatures up to 560°C. It appeared that the hydroxyapatite matrix of the bone char and the dispersed carbon therein each had its own optimum regeneration temperature. A working hypothesis was postulated to explain the steps in kilning: first, adsorbed organic matter is pyrolyzed at about 300°C to form an inactive coke; then at 500°C, the inactive coke decomposes to form an adsorbing residue. This model is still a valid explanation for more recent research findings by other workers on char.

Sugar Losses

In the last quarter of 1955, a report (19) was issued giving preliminary data for the amount of sucrose retained by bone char that has been used for sugar adsorption. The method used for sucrose determination in this report was considered somewhat crude, and shortly a much more refined method, based on pyrolysis, was developed. The new procedure showed that as much as 0.4% of sucrose per weight of char may be retained by good quality char. This information has helped fill in the gap of unknown sugar loss.

Sugar Color

In addition to investigations on bone char the Project also conducted studies on the nature of the impurities in sugar--a logical move since their removal is the basis of the whole adsorption process (10, 17, 22, 23, 29, 31).

Color, its measurement and its composition are reported on at length many times (4, 5, 16, 18, 20, 21, 25, 30, 34). In the usual progress of analytical development, the work started with "brown bottle" based methods and graduated to advanced techniques. Along the way much was learned about the properties of color. The delineation of the nature of color was not worked on as such by the Bone Char Project, but in recent years has been a major objective of the Cane Sugar Refining Research Project.

First, it was necessary to distinguish between color and turbidity since both contribute to the appearance of sugar in solution. With the sugar world finally convinced that some of what was generally called color was actually turbidity resulting from poor filtering, and not color resulting from poor adsorption, the subject started to make progress (4, 5, 11, 12, 21, 24, 26, 28, 35, 39). The Project introduced to sugar technologists the idea of the combination of color and turbidity as attenuation, although that term has not stuck. Technical Report 65 issued in March, 1962 (4) sums up the work on color and makes recommendations for a precise method, using a spectrophotometer, for measuring color (3, 37). Procedures later adopted by ICUMSA (33) follow this method closely except that readings now are made at 420 nm rather than at the 365 nm recommended by the Project (6).

DH Test

For many years the lye test was the sheet anchor for the sugar technologist seeking to evaluate the efficiency of his char kilns. Occasionally a little basic lead subacetate was added to see if the very light char particles had been overburned (38). With the advent of the pH electrode, the pH of the water extract of reburned char was considered to give better information. Then in 1959 a new test, DH, made an appearance. Originally proposed by J. Guerin (32), it stood for "degré hydrotimetrique" but the Americans soon renamed it "degree of hardness" (27). DH is considered to be an indicator of the ability of a regenerated char to remove calcium and magnesium ions from sugar liquors passed over the char, particularly for the first five displacements. Recently this measurement has been incorporated into Tate and Lyle's proposed Index of Performance (1).

Excess Polyvalent Anion Measurement

There was a time in the sugar industry when EPA did not stand for Environmental Protection Agency; instead, it had the equally challenging but happier connotation of "excess polyvalent anions". Toward the end of the Project a report was issued on "Ionic Interactions With Sugar Colorant During Char Filtration" (9). Parts of this report were also presented by Dr. Carpenter at the 7th Technical Session on Bone Char (8). The information presented in these reports began to elucidate the mechanism whereby colorant is removed from sugar liquors by bone char. The concept is neatly summarized by the following quotation from the abstract of the Project Report 69 (9):

"The results of many measurements were correlated through a quantity termed "excess polyvalent anions" (EPA). The EPA, expressed in concentration units, can be evaluated from the following relationship:

$$EPA = TA - Cl - Ca = SO_4 + OA - Ca$$

where TA is total anions, Ca is measured by the EDTA titration, and OA represents the other anions, i.e. $TA - Cl - SO_4$.

A reaction mechanism is proposed to correlate the unknown facts in which it is postulated that the anionic and molecular colorant are partly interconvertible. Part of the mechanism of colorant removal is the adsorption of the Ca-colorant as an ion pair and part as an ion exchange of other anions for the colorant anion.

An anion adsorption series is proposed and the following sequence of ions appears valid for the adsorption process on bone char:

$$HPO_4 > CO_3 > SO_4 > OA > a^{*-}$$

Where a^{*-} represents anionic colorant."

Tests were conducted in which small amounts of solutions of either calcium chloride or potassium sulfate were added to sugar solutions containing colorant made by the thermal degradation of sugar solution. When passed over char, "calcium ions from calcium chloride improved color removal whereas the sulfate ions from potassium sulfate inhibited color removal."

This reported research generated a brief flurry of activity at many refineries to experiment with the addition of calcium chloride to sugar liquors prior to char filtration. Some initial successes were reported but these were generally not followed up because of the difficulty of measuring all the secondary reactions in an operating refinery. So one of the most promising leads postulated by the Project has gone largely unused, even though the concept helps to explain why different cargoes of raw sugar decolorize differently although their analyses by standard sugar refinery techniques are almost identical, and the condition of the refinery char house is the same between cargoes.

CLOSING OF BONE CHAR PROJECT

The last report of the Project, on the role of carbon dioxide on char performance, dated July 26, 1963, marked the end of activity (7). There remains a haunting question: why? Why was a research project that had built a skilled team, developed the necessary analytical methods, built good experimental models and was presenting new and useful information cut down in its prime? The government had decided against continuing sponsorship of this type of cooperative research but the total cost, if transferred elsewhere, to a university, institute or private laboratory, would hardly have been significant when spread over many supporting members. There was at that time a spirit of anti-research common to the industrial world although the presence of Sputnik whirling around the world was soon to initiate a great revival in physical research, most of it in physics and the space sciences. The answer must lie within the inability of the technologists of the Project member companies to convince their corporate superiors that research paid off in bottom line dollars--to use the jargon of the day.

Eventually, a way was found to keep the sugar research flame burning through the establishment of the Cane Sugar Refining Research Project, under the leadership of Dr. Frank Carpenter, at the Southern Regional Research Center. Often quoted is the remark that those who don't study history are doomed to repeat it. Perhaps that is the lesson for us here today: to use the results of the research that will be reported, and to give Frank guidance to the areas where meaningful answers are needed.

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DISCUSSION

R. S. PATTERSON (California and Hawaiian): How much of the work done by the Project applies to granular carbon as well as to bone char?

K. R. HANSON: Some of the work can be applied to activated carbon regardless of origin. Some of these reactions are due to the carbon-phosphate system

of char. We know that phosphate in the carbon takes out ash. Granular carbon certainly takes out a lot of color, and powdered carbon also takes out color. I will fall back on the old sugar man's attitude that carbon takes out color and phosphate takes out ash, and that there is a big interlock between the two.

F. G. CARPENTER (C.S.R.R.P.): The concept of excess polyvalent anions (EPA), which was brought out by the B.C.P.[1], indicates that it is not carbon on one hand and phosphate on the other, but that the carbon somehow ties the calcium and color together. Both are adsorbed on the char at the same time.

K. R. HANSON: EPA, which came very late in the life of the Bone Char Research Project, was probably one of the most interesting concepts and certainly deserves a lot more research and thought. There is a system in bone char which you don't get in pure carbon or pure phosphate. Of course, the work that you and others have done now in distinguishing among colorants goes a long way here, because we know now there is ionic color and non-ionic color. There is also the problem of color and turbidity. In addition, you get reactions that are both color reactions and ionic reactions. So I hope that someone picks up and continues to run with the EPA concept to work further on some of these problems.

F. G. CARPENTER: May I ask if anyone actually uses the concept of EPA in their daily operations?

K. R. HANSON: It is pretty much in disuse these days, although we know that as different raw sugars from different countries come through, some sugars with the same apparent color react very differently on bone char. These may be high or low ash sugars. This certainly gives indications that EPA is in effect in the char house. Jim Culp did experiments by actually adding calcium chloride to liquors at one time, trying to reach a balance, but this work was never brought to a fruitful conclusion.

F. G. CARPENTER: In certain refineries I have seen bags of calcium chloride that were open for use so they must have been used for something. Essentially, the calcium chloride is only needed when the sulfate is high, and that isn't so for every raw sugar.

K. R. HANSON: This has been largely abandoned at the moment.

F. G. CARPENTER: The results can be spectacular: color remaining can be decreased by a factor of ten. Added calcium will greatly improve a very poor sugar, but not every sugar shows that spectacular effect.

K. R. HANSON: Also, there are other effects in the refinery which to some extent cancels out the use of added calcium. I feel that the EPA was a very valid concept, and is certainly open to more research.

F. G. CARPENTER: What, in your and others' opinion, was the most valuable practical contribution of the Bone Char Project?

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[1]Carpenter, F. G., Larry, D., and Deitz, V. R. 1962. Bone Char Res. Rep. 69, 34 pp.

K. R. HANSON: I think the most valuable part of it was that the industry learned that there are things to be discovered by cooperative research. When you read the various reports you see many, many, concepts which have worked their way into the consciousness of sugar refining; for example, people have largely accepted the concept of the two-temperature char washing that is in use in many places. Very early workers talked about discarding bone char at 80 lb/cu ft, whereas people have now accepted the fact that bone char does have pores that fill out mostly with calcium sulfate, and that you've got to wash properly. This Project came in an era of developing instrumentation for refinery use. It started almost in the litmus paper era.

F. G. CARPENTER: George Meade used to say, and I am sure that he would still say today, that the most important advance in the sugar industry was litmus paper.

K. R. HANSON: Probably true, and we've just made the basic idea a little better. That went a long way; at least you learned the idea that alkalinity and acidity played an important part. I think the concept of research has a role to play in any industry, particularly in the sugar industry.

A. M. JAMES (Tate and Lyle): We were talking about ways to revivify char. I wonder if anyone has any views to express on the differences between pipe kilns and multihearth kilns. Normally, nowadays any new kiln installed is of the multihearth type. Does everyone think the burning is actually better in the multihearth kiln, or were there any benefits to the old pipe kilns? Clearly one is likely to get a lower carbon content with the multiple-hearth kiln.

R. S. PATTERSON: We have three grades of char and on one occasion our poorest had really gone downhill: it was just about 17% carbon. Color removal and so on was pretty terrible. We put in a multiple-hearth furnace, and the results were dramatic: the improvement was at least 50% on that char. This is rather an extreme case.

K. R. HANSON: Did you run the multiple-hearth furnace with excess oxygen?

R. S. PATTERSON: Yes, oxygen was between 4% and 6%. That was able to bring down the carbon content to between 10% and 11%, and also seemed to burn out some of the material that had been in the pores for some time.

K. R. HANSON: You can use a multiple-hearth kiln as a decarbonizer. I wonder if many people have every seen a Weinrich decarbonizer. I burned one up once--that must have been about the last one in the industry. The char was heated in the presence of open atmosphere to a dull red heat, while it trickled down a big cast iron drum, like a granulator without any internals.

F. G. CARPENTER: The decarbonizing problem is discussed in Bone Char Report 68[2,3]. There are two types of carbon in char: there is the freshly

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[2]Carpenter, F. G., and Deitz, V. R. 1962. Bone Char Res. Rep. 68, 24 pp.

[3]Carpenter, F. G., and Deitz, V. R. 1961. Reaction of oxygen with unkilned bone char at low temperatures (300°C). Proc. Tech. Sess. Bone Char 7: 237-57.

adsorbed carbon from the last cycle, and there is the carbon that has been burned on with repeated burning. Report 68 discusses the dependence of the rate of reaction on temperature and oxygen concentration for the different types of carbon. If a Herreshoff kiln is altered slightly by opening one door, so that there are two zones, there will be a higher oxygen concentration with a lower temperature in the upper part, and a lower oxygen concentration with a higher temperature in the lower part of the kiln; then, control is obtained on the rate of burnoff of both the freshly adsorbed and the burned-in carbon. However, no one seems to do this in actual practice.

K. R. HANSON: One thing I didn't say and meant to say was that very early in the project Dr. Barrett persuaded the Bone Char Research Project that Synthad was bone char[4]. He was one of the leading lights in the whole Project, and he was most successful in proving that Synthad was bone char.

F. G. CARPENTER: Yes; in his original paper he had everything within a fraction of a percent the same between Synthad and bone char, for about a dozen properties[5].

R. S. PATTERSON: To return to the Weinrich decarbonizer: we had one at Redpath in Montreal that was used for decarbonizing, and it did such a good job that, in considering design for a new char house, we wanted to incorporate a rotary drum regenerator. At that time everyone was in favor of the rotary hearth kiln over the pipe kiln.

K. R. HANSON: One argument in this area is the Weinrich decarbonizer versus the Niese box. The Niese box is nothing but an open, slotted, cast iron piece between the retort and the cooling pipe. There is a long discourse in one of these papers on the role of decarbonizer versus Niese box[6].

G. W. MULLER (S.I.T.): I'd like to give some background on bone char revivification. We did many experiments in the 30's with the old tubular and oval steel retorts, and one of our aims was, antedating the work of Carpenter, to get the zones of revivification. We attempted to get rid of the vapors off char that may condense back on as the char cools. In some systems the char retorts and coolers were sealed, and there was no way for vapors to escape. Many of these vapors go down into the cooler and are condensed. These are the char odors in the first liquor off char. We set up one kiln especially to sweep steam through. The char cascaded from a Niese box, and was in an atmosphere of steam. We did a very good job with the steam--there was no char flavor in liquor coming off. We did, in fact, the work that you might get in the rotary kiln. This work was never reported.

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[4]Pennington, N. L., Beal, C. W., Heath, W. D., and Morrison, D. C. 1955. Plant scale evaluation of Synthad C-38. Proc. Tech. Sess. Bone Char 4: 195-246.

[5]Barrett, E. P., and Brown, J. M. 1949. Some experiments with synthetic granular adsorbents for sugar refining. Proc. Tech. Sess. Bone Char 1: 198-240.

[6]Deitz, V. R. 1952. Bone Char Res. Rep. 27, 24 pp.

F. G. CARPENTER: In Bone Char Report 66[7,8], the effect of CO_2 on the pH of the char was measured. CO_2 adsorbs on revivified char as it cools down. If the kiln gases go in the wrong direction, the CO_2 gets back on the char, and that acts just like an acid. The pH of the first liquors off char is very sensitive to CO_2. In a standard pipe kiln, if some of the gases that are given off in the hot zone go out downward into the coolers, they are readsorbed and this gives a miserable job of regeneration.

W. W. BLANKENBACH: I think that we owe a debt of gratitude to Ken for reviewing this very important work. I personally think that the Bone Char Research Project was one of the best things the sugar industry ever did. It was, I fear, fated from the beginning to get rather meager support from management, who expect a high return on every research dollar. This project marked the first time that the sugar refining industry had ever gotten together and shared some of their knowledge. Refining had, historically, been a most secretive sort of business, and when this Bone Char Project was set up, it brought together the technical staffs of practically all the refineries in North America. For the first time we were able to exchange technical information, and this has developed into the very free exchange that we now have in the industry. That has benefitted the industry tremendously; in fact, it may be the most useful contribution of the Bone Char Project.

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STARCH: ITS OCCURRENCE, IMPORTANCE, AND REMOVAL IN SUGAR MANUFACTURE

By John B. Alexander[1] and M. Matic[2]

(Presented by M. Matic)

ABSTRACT

Starch in sugarcane occurs in the form of insoluble granules which are extracted with the juice in the milling process. The action of heat and lime solubilizes the granules, and most of the starch passes into the raw syrup. In relation to other impurities, starch has a greater tendency to be included in the raw sugar crystals during boiling and hence get selectively transferred to the refinery.

Carbonatation refineries have recognized that the action of starch in affined raws can be responsible for producing very poor filtering calcium carbonate slurries, severely reducing throughput, and focusing attention on filterability tests. Recent studies have suggested that it is the amylose fraction of starch acting as a protective colloid and coating the surface of the growing carbonate crystals which suppresses agglomerate formation resulting in low average particle size and poor filtration. For this reason the starch (or amylose) content of a raw sugar is considered to be far more meaningful than the simple filterability type test.

Various methods of starch removal and their effectiveness are discussed. Details of the results achieved by the use of bacterial amylase in raw sugar factories in South Africa are given.

The difficulties of obtaining pure cane starch and a method of analysis which is both reliable and meaningful for use in the sugar industry are mentioned.

INTRODUCTION

Starch is found to varying degrees in all commercial varieties of sugarcane. The failure of Dutt and Narasimhan (21)[3] to detect starch in some varieties is probably due to the use of analytical techniques insufficiently sensitive for very low concentrations.

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[3] Numbers in parentheses refer to items under "References" at the end of this paper.

Cane starch, like other starches, consists of two major polysaccharide fractions, which in themselves have a range of molecular weights. The straight chain or amylose fraction has different properties from the branched chain or amylopectin fraction. It should always be remembered that not only can some of the properties of "cane starch" change during its journey through the manufacturing process, but also that the ratio of amylose to amylopectin can vary.

Pure cane starch has, to our knowledge, not been isolated in an unchanged form (6). Since starch is a high molecular weight polysaccharide and as such, the cause of processing problems, starch has undoubtedly had more attention focused on its role in sugar manufacture than it has on its role in sugarcane physiology. As early as 1926, Haddon (25) in Natal reported abnormally high viscosity massecuites produced from the cane Uba, which was then the major variety grown. To his credit, Haddon patented (26) a process for using high temperature enzymes, much along the lines adopted in recent times, for reduction of cane starch in sugar manufacture.

DISTRIBUTION OF STARCH IN SUGARCANE

Starch is distributed throughout the cane stalk, mainly in the form of small granules in the size range 2 to 10 microns (average 5 microns). Wood (45) reports a higher concentration of starch in the top portion of the cane stalk and a 3.5 to 4.0 times greater concentration of starch in nodes than in internodes. Not all the starch is present in the form of granules, and both Wood (45) and Prince (37) have found a variation in the amount of soluble starch in cane juice ranging from trace up to as much as 25% of the total starch.

There is undoubtedly a large variation in the ability of different varieties of sugarcane to produce starch. The varieties Uba and NCo 310 are well known for their high starch contents. It has been suggested that it is the varieties with "primitive" Saccharum species in their ancestry that are able to accumulate starch (1).

Though it is generally accepted that the same variety of sugarcane grown in different areas will vary in its starch content, the extent to which individual factors are responsible for this variation is not clear. Before the introduction of special starch reducing processes in raw sugar manufacture, there appeared, in general, to be less starch in sugar originating from the tropics than in sugar from more temperate climates. Among the many factors other than climate reported to affect starch level in sugarcane are soil type, soil pH, and potash content of the soil (1).

STARCH IN THE RAW FACTORY

The normal milling process releases starch into the extracted juice stream mainly in the form of insoluble granules. In cases where diffusion is practiced, the quantity of starch entering the juice stream has been found to relate to the temperature to which the granules are subjected during the diffusion process (14, 24). This observation can be explained because the starch granules burst as the gelatinization temperature is reached in the diffuser. Juice from most cane diffusers can therefore be expected to contain a greater percentage of starch in solution than in the case of milling.

The action of heat and lime in the common raw sugar house clarification processes renders most of the starch granules soluble, so that the major part of starch in mixed juice passes into the clarified juice.

Tests carried out by the Sugar Milling Research Institute (5) showed that more than 80% of the starch in the mixed juice passed into the clarified juice, whether simple defecation or sulfitation was practiced. Natural enzymes in the cane juice are inevitably responsible for the removal of some starch in most raw factories.

Industry, and in particular the organic chemical industry, has long recognized the process of crystallization as one of the most powerful tools for product purification. In sugar manufacture the final product has invariably passed through at least one crystallization stage where the reduction of highly soluble nonsucrose compounds is often taken for granted. Unfortunately, some of the high molecular weight impurities, such as starch and other gums, show a much greater tendency to cocrystallize with sucrose and pass on with the sugar than to be eliminated with the mother liquor or molasses. Calculations made on South African sugars in 1957, when no starch removal processes were used, show that the ratio of impurity entering the raw crystal as opposed to that ending up in molasses was more than ten times higher for starch than for reducing sugars. The inability of the affination station to achieve an effective starch reduction is yet another manifestation of the selective inclusion of starch within the sugar crystal itself.

When the partial remelt system (43) was introduced in South Africa to improve raw sugar quality, the results with respect to starch reduction were disappointing, even though the main offenders, all C-sugar and much of the B-sugar, were being remelted. The starch was found to be selectively included in the A-sugar crystals during recrystallization.

STARCH IN THE REFINERY

In a carbonatation refinery, the filtration rate of a carbonatated slurry is of great importance, as it determines to a considerable extent the throughput of the refinery. Experience has shown that various raw sugars filter differently in the refinery and considerable work has been carried out in the past in order to establish which impurities in raw sugar are responsible for this behavior (2, 3, 16, 17, 19, 20, 33, 48). Studies have been made either by measuring resistance of a refinery filter cake when a specific sugar was processed, or by comparing the analysis of raw melt with the refinery performance. In this way, gums, wax, phosphates, silica, and, in particular, starch were at various times proposed as the cause of poor filterability.

As, for obvious reasons, it was difficult to establish a direct correlation between these impurities and the performance of sugar in the refinery, various laboratory filterability tests were evolved (3, 23, 34, 36) and used as a yardstick to measure the influence of various impurities on filterability of sugar. All of these tests were based on the measurement of a volume of filtrate obtained by passing a raw sugar solution under standard conditions of Brix, temperature, pH, pressure, and time through a suitable filter medium, such as membrane or kieselguhr. The results of these tests were generally

adopted as an indication of filtering quality of a raw sugar, and the tests became a part of quality specification in many countries (e.g., U.S.A. Contract No. 10). Unfortunately, the introduction of this test has, instead of clarifying, confused the issue. Although it has been generally accepted that, in carbonatation refineries, starch is the most important filter-impeding impurity, Alexander (3) has shown that there is little or no correlation between the laboratory filterability and starch content. A similar conclusion was reached by Yamane (29, 46) who also found that a very high correlation (-0.92) existed between the results of a filterability test and insoluble matter in raw sugar (47). This was confirmed by Tu (42). Yamane furthermore demonstrated that when a filterability test was carried out on a carbonatated slurry instead of a sugar solution, a close relationship was obtained between filterability and sugar-starch content (correlation coefficient 0.95). Finally, it was possible to demonstrate (28) that while results of a laboratory filterability test showed low concurrent reliability when compared to refinery filtration data, results of starch tests were much more reliable.

These seemingly contradictory findings were explained by Murray (31) who recently studied the influence of suspended matter and starch on both the laboratory filterability test and the filterability of carbonatated slurry. Using the same sample of raw sugar, from which various amounts of insoluble suspended matter had been removed by membrane filters of decreasing pore size, he was able to show that there is a straight-line relationship between the increase in laboratory filterability and the removal from the sugar solution of suspended matter of diameter greater than 0.5 μm. He also demonstrated, by analyzing the sugar solution before and after the filterability test, that no starch was removed during this process. He further showed that there was no change in laboratory filterability of a sample of the same sugar from which various quantities of starch had been removed by means of enzyme.

However, when the filterability of a carbonatated slurry produced in a laboratory under steady state conditions was compared to either total suspended matter (0.2 μm-40.0 μm) or total starch in sugar, very good correlations were obtained (both correlation coefficients were -0.98). However, the magnitude of the influence of starch on filterability was found to be very much greater. In these experiments the filtration rate of the carbonatated liquor was based on the incremental rise in filter cake resistance with time, as discussed by Bennett (8). This author was also able to demonstrate that the type of calcium carbonate precipitate formed during the reaction not only varies with the raws used but is also fairly characteristic for any particular raw sugar. Using electron microscopy, Murray (32) showed that the precipitate resulting from the laboratory processing of a high starch raw sugar (325 ppm) was characterized by a poor degree of agglomeration. The particles were small and regular in shape, thus allowing a close packing arrangement. In contrast, the precipitate derived from a low starch raw sugar (80 ppm) consisted of large, well-formed agglomerates of irregular shape. When packed, these presented a relatively large leakage diameter. The recorded carbonatation filterabilities (f_c) for these two sugars were respectively 27 and 65, on the Bennett "filterability index" scale (8, 9) where a laboratory "carbonatation filterability," f_c, is defined as

where "m" is the slope of a graph of the reciprocal of the filtration rate versus the cumulative volume of filtrate collected.

This work confirmed that starch plays a significant role in the carbonatation reaction and explained the reasons for the previously observed discrepancies. In a laboratory filterability test, the pore leakage diameter of the filter bed is determined by the characteristics of the filter medium used, and is independent of the starch content of sugar. In contrast, in the carbonatation filterability, the properties of the filter bed are dependent on the characteristics of calcium carbonate crystals formed during the reaction on which starch has a significant influence. Murray concluded from these experiments that while the laboratory filterability test may be a fair measure of suspended matter present in raw sugar, the test can give only a poor and even a misleading indication of how the sugar will behave in a carbonatation refinery.

Location of Starch in the Calcium Carbonate Crystal

A closer examination of the calcium carbonate precipitate produced in the laboratory revealed that starch is found both within the crystal and on its surface. The surface starch could be successfully removed by treatment with hot water, while starch held within the crystal was only released by slowly dissolving the crystal in cold hydrochloric acid. The surface starch was shown to be composed predominantly of amylose whereas starch included within the crystal matrix was very largely amylopectin. This suggested that the two major starch components act independently and influence the crystallization process in different ways. By adding pure amylose or pure amylopectin to a starch-free raw sugar, and repeating carbonatation and separation treatments, the hypothesis was confirmed. It was further established that the calcium carbonate precipitate produced in the presence of pure amylose was composed of well defined single crystals with practically no agglomeration. Carbonatation filterability of such a sugar gave an f_c value of only 20.

It would appear, therefore, that amylose is the main filtration-impeding impurity present in raw sugar. It can be calculated from Murray's data that on a weight for weight basis, the influence of amylose on carbonatation filterability is approximately ten times greater than that of suspended matter, six times that of amylopectin and more than twice that of total starch.

Murray explained the mechanism of the influence of starch on carbonatation filterability in terms of the properties of the two starch components. Amylopectin which contains phosphate groups is negatively charged, and interacts with unfilled anionic sites (effective positive charge) on the growing crystal surface. The molecule is immobilized and included in the crystal matrix. Consequently, it has little influence on the nature of the crystal surface, which is the main factor in determining the degree of agglomeration. Amylose on the other hand is electrically neutral. It accumulates on the surface of the growing crystal and acts as a protective colloid, thus effectively suppressing the formation of agglomerates. This, in turn, produces a calcium carbonate precipitate of low average particle size and poor filter leakage diameter.

Filterability and Refinery Performance

Finally, an attempt was made by Murray to correlate these findings with the actual raw sugar performance in the refinery. The results of this investigation are presented here.

For this purpose the conditions of laboratory carbonatation used in earlier work were altered to resemble more closely those of refinery operation. Reaction time was reduced to 60 min and technical grade lime was used (0.6% CaO on solids).

A comparison of the filtration rate of laboratory-produced carbonatation slurry with refinery-produced slurry from the same raw melt liquor was first undertaken to assess the relationship between the two processes. A composite sample of refinery raw sugar, before it entered the melter, was collected over a period of 2 h. Allowing a time interval of 1.5 h as average residence

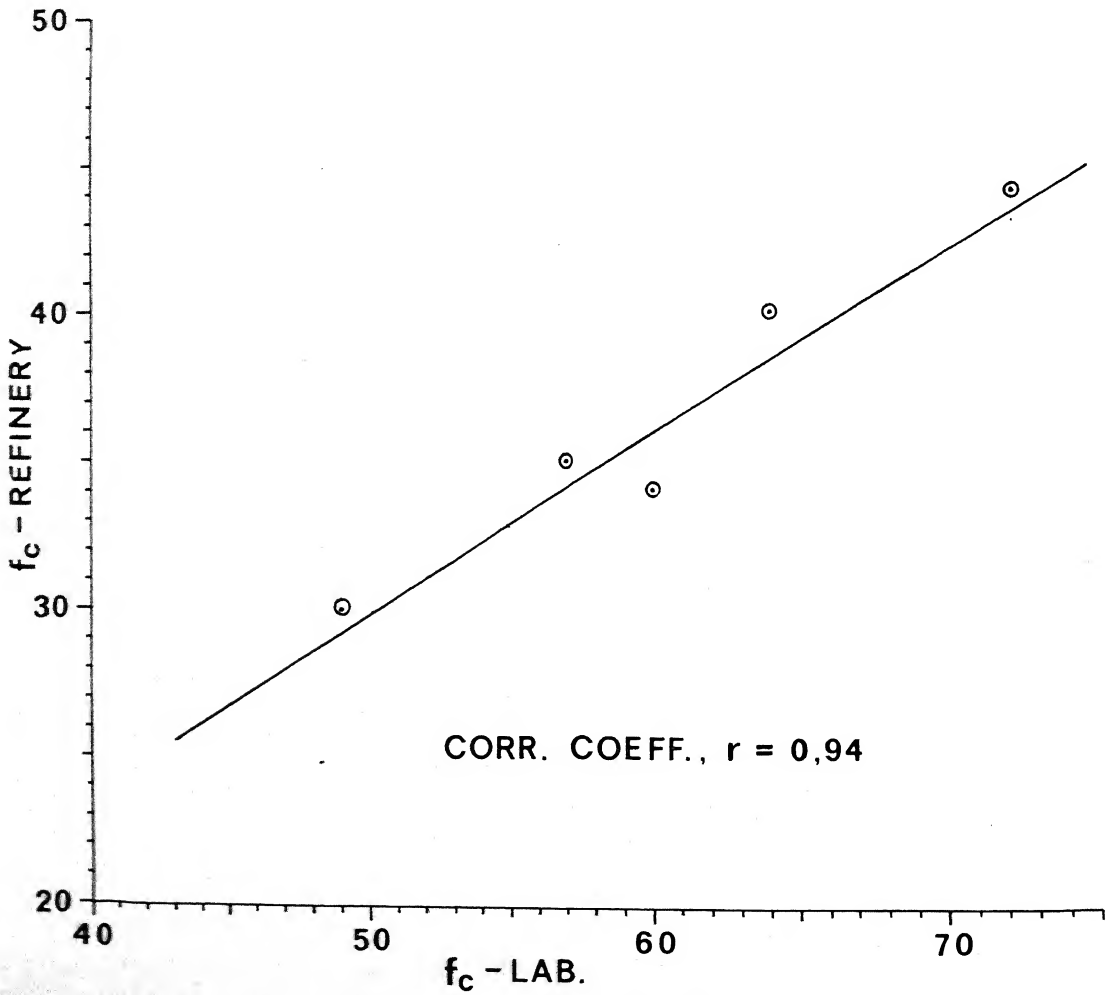

FIGURE 1—Correlation between refinery and laboratory data.

time between melter and filter station, a composite sample of carbonatated liquor was withdrawn again over a period of 2 h. This sample was filtered on the laboratory filter apparatus and the rate recorded as the refinery filterability, f_c-ref. The raw sugar sample was then melted and processed in the laboratory apparatus under the conditions described above. The liquor produced was filtered and the rate noted, as f_c-lab. A typical relationship for a selection of raw sugars is given in figure 1.

The high correlation coefficient ($r = 0.94$) between laboratory and refinery filtration rates is indicative that the laboratory apparatus is capable of producing a slurry with essentially similar properties to the refinery slurry, with the exception that the filtration rate is, on average, 1.5 times greater. This difference is probably due to more favorable pH conditions during crystal growth in the laboratory reactor. Similar differences were reported by Bennett (10).

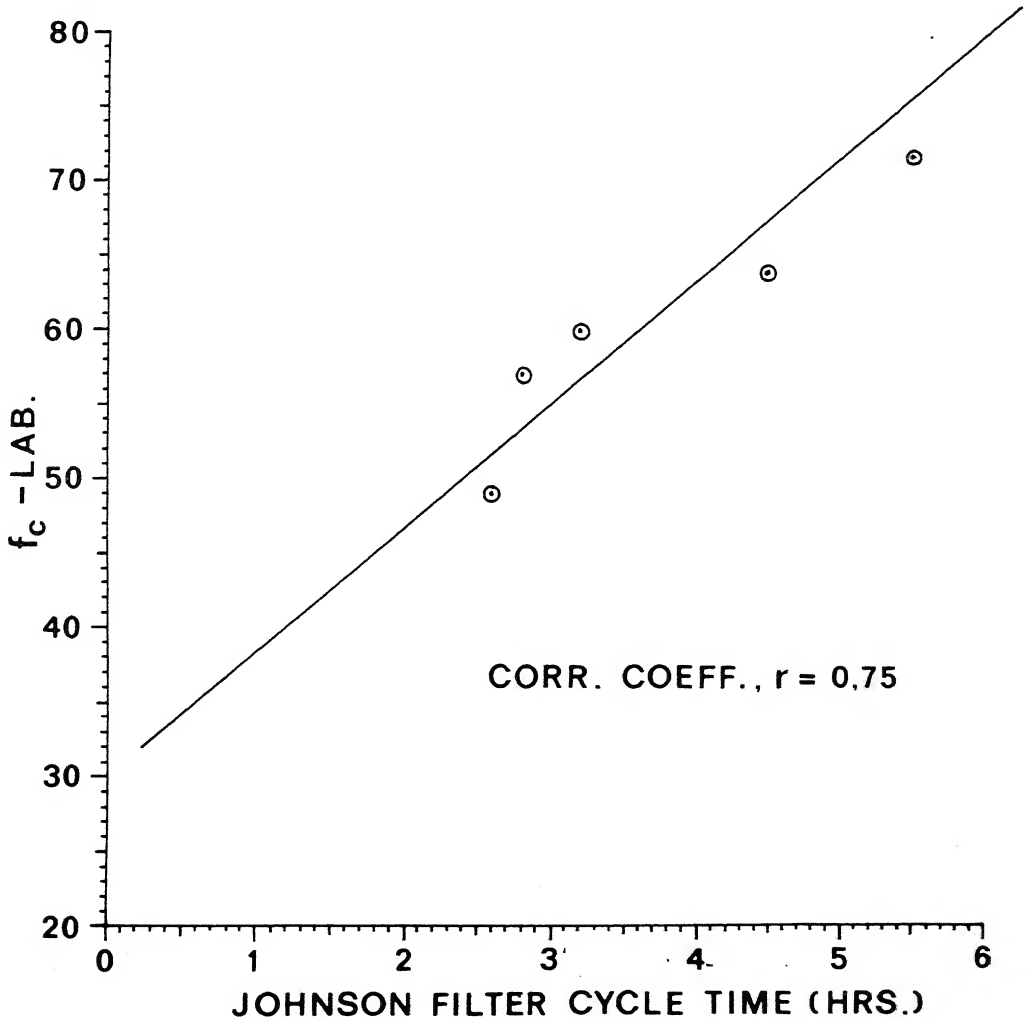

FIGURE 2--Correlation between refinery filter station data and laboratory filtration data.

The quantity, f_c-ref, is essentially a laboratory assessment of refinery filtration rate. A direct measure of filter station throughput, which could be related to sugar quality, is far more difficult to achieve since other factors serve to alter the filter performance, e.g., change in Brix of melt, change in % CaO on solids, etc. However, by taking the average cycle time of one filter over the period covering the experimental runs it was possible to measure the correlation between this factor and laboratory filtration rate, f_c-lab, as shown in figure 2. The resulting correlation coefficient of r = 0.75, although much less significant than the previously discussed figure, again validates the use of the laboratory apparatus in appraising refinery filtering performance.

Next, the comparative influence of starch and the amylose fraction of starch on the magnitude of the f_c-lab results was assessed using the refinery raw sugar samples. Starch in raw sugar was determined by the standard method (30) and the amylose content was calculated according to the amperometric technique at present under development at the Sugar Milling Research Institute and described elsewhere in this paper. The results show that the correlation coefficient for amylose (r = 0.84) is somewhat higher than that for starch (r = 0.79) in agreement with the earlier conclusions. This suggests that the determination of the amylose content of raw sugar, rather than the starch content, may give a more accurate prediction of the filtration properties of carbonatation slurries made from the sugar. Some thought could perhaps be given to introducing this measurement of sugar quality into specifications of raw sugar.

The discussion of starch influence on refining has so far been concerned with the carbonatation process for the simple reason that most South African export sugar goes to this type of refinery. Initially, the main effort was, therefore, directed at understanding the mechanism of starch interference in this process. However, some preliminary experimental work has indicated that starch also plays a role in the phosphatation-flotation process. When starch occurs in substantial quantity in raw sugar (e.g. >350 ppm) it was observed that the rate of calcium phosphate floc formation and its subsequent flotation rate were affected. Physical properties of the floc, e.g. compressibility, also change markedly. It is, however, not yet known what role, if any, the amylose fraction of starch plays in these processes, and quantitative assessments of the various factors influencing phosphatation are at present being studied in our laboratories.

STARCH DETERMINATION

There are numerous methods for starch determination (27) but the one generally used in the sugar industry is based on the measurement of the intensity of color produced by the starch-iodine complex. The existence of many versions of the original Balch (7) method is in itself an indication that the colorimetric method is not entirely satisfactory. In a recent study (30), in which various sources of interference have been examined, it was demonstrated that the results depend on the starch used as a standard, the concentration and pH of the extracting calcium chloride solution, the amylose-amylopectin ratio of the starch in the sample, and the wavelength at which the color is measured. Since the amylose-amylopectin ratio of starch varies not only with

the age and variety of cane (15) but also from one sugar sample to another (13), depending apparently on the conditions in a particular factory, the colorimetric methods can give only an approximate value of the starch content of sugar. Furthermore, little indication can be obtained about the amylose content of starch by this method.

It was suggested earlier in this paper that amylose rather than starch in sugar should be measured in order to assess the filtering quality of sugar, and preliminary work to establish a suitable method of analysis is underway in the S.M.R.I. laboratories (18). An amperometric titration method similar to that described by BeMiller (44) is used for this purpose. Since the iodine binding power of amylose from starches of various origins differs little (19.0 to 20.6 mg/100 mg) no standard is required in this determination, which is a great improvement over the colorimetric methods. Although reproducibility of the amperometric method appears to be acceptable, with duplicate analyses differing by less than 3 ppm, the sensitivity is still not satisfactory. In order to detect 10 ppm of amylose, a sample of 100 g of sugar is required. This makes the method, in its present form, somewhat cumbersome. Nevertheless, the analyses of several raw sugars by this method are presented in table 1 together with starch values obtained using the colorimetric method. From this, it is apparent that misleading conclusions regarding the filtering quality of sugar can be drawn if starch figures alone are considered. For example, sample D should probably filter better in the refinery than sample E, although the latter contains less starch.

TABLE 1--Amylose and starch in raw sugars

Sample	Amylose(ppm)	Starch(ppm)(30)
A	18	130
B	24	150
C	15	45
D	<10	120
E	24	80
F	36	170

METHODS OF STARCH REMOVAL IN SUGAR MANUFACTURE

Basically there are four avenues of attack which have been used to reduce starch in sugar manufacture. They can be broadly enumerated as follows:

1. Mechanical removal of the starch granules from cold raw juice.
2. Cold flocculation and removal of starch granules with the floc.
3. Adsorption of starch on suitable adsorbants such as vegetable carbon.
4. Degradation of starch molecules to smaller and innocuous molecules.

Mechanical Removal from Cold Juice

The colloidal and waxy materials usually present in mixed juice render it an extremely difficult liquid to filter and in consequence, attempts at mechanical purification have been based almost exclusively on the use of centrifugal force. Only granular and no soluble starch can be removed by centrifugation, which must be carried out prior to heating. Tests carried out on a factory scale in South Africa in 1958 (4), using a QX 210-20 De Laval centrifugal separator, showed that 80% starch removal could be achieved. Erosion of both the centrifuge bowl and discharge nozzles by sand in the juice curtailed the tests, and subsequent tests with a machine modified to reduce erosion were unsuccessful due to low efficiency in starch removal.

Cold Flocculation

An acid preclarification process advocated by Dymond (22) undoubtedly removed starch granules, but was never put to commercial use, mainly because of the difficulty in separating the settled precipitate from associated clear liquor.

The Rabe'(38) process was used by at least four factories, to manufacture more than 200,000 tons of low starch raw sugar in South Africa. Basically, it relies upon the vacuum flotation of a calcium phosphate floc to entrap starch granules at a temperature below 70°C. Difficulty in controlling the flotation and postflocculation at the evaporation stage coupled with high chemical costs led to the abandoning of the Rabe' process in favor of the use of high temperature enzymes in South Africa. Prince (37) has attributed unusually low starch removals, which sometimes occur in the Rabe' process, to the presence of increased amounts of soluble starch in the mixed juice. With few published figures available, it seems doubtful whether any significant removal of soluble starch can be expected in most cold flocculation processes.

The U.S.D.A.'s Agricultural Research Service has carried out considerable work in an effort to find a viable process to permit the manufacture of sugar from sweet sorghum juices. Starch can constitute up to 4% of solid matter in sorghum juices and at these levels poses a major problem during crystallization, if not removed. Smith, of the U.S.D.A.'s Agricultural Research Service, has several patents (39, 40) for the removal of starch from sweet sorghum juices which utilize defecation and polyelectrolyte flocculation. Removals of up to 97% starch are reported.

Adsorption

The authors know of no raw sugar producer who has used an adsorbant for starch reduction. However, the refining process practiced at Illovo in the early 1950's achieved a starch-free refined sugar in spite of melting raws very high in starch. Alexander (2) attributed this to the high percentage of vegetable carbon used (up to 1% on melt) by that refinery. He showed in laboratory tests that 0.5% carbon was sufficient to achieve a starch-free liquor. Bone char was found to give a negligible starch removal.

Degradation of Starch

Since sucrose is more prone than starch to hydrolysis by mineral acids it is not feasible to consider their use for the removal of the trace quantities of starch present in sugar juices.

Haddon (25) was probably one of the first to treat cane juice with enzymes when he used an enzyme he called Ubase to reduce the starch in the notorious Uba cane juices.

Tests using malt enzymes carried out by Boyes (11) showed this type of enzyme to be entirely uneconomical for the purpose of starch reduction in raw sugar manufacture.

Nicholson and Horsley (35) in Australia showed that by the careful control of pH and temperature, the natural enzymes present in cane juice could be utilized for hydrolyzing an appreciable amount of starch in mixed juice. Although the natural enzyme process has been used in a number of countries to reduce effectively the starch intake in raw factories, it suffers from the disadvantage of varying enzyme content of juices and consequent control problems with respect to both starch hydrolysis, and the hydrolysis of sucrose by invertases in the juice.

In South Africa, experience both in the laboratory and in the factory has been that a removal of starch in excess of 50% was seldom achieved. This has led to the abandoning of natural enzymes in favor of controlled addition of enzymes derived from bacteria. With the advent of the extensive use of bacterial α-amylases in the textile and detergent industries, effective enzymes became commercially available for the reduction of starch passing into raw sugar. The higher operating pH and thermal stability of the bacterial enzymes allows their use in clarified juice or sirup (12). Furthermore, the high sucrose concentration protects the enzymes at higher temperatures allowing the efficient use of bacillus subtilis derived enzymes at temperatures up to 80°C, while those derived from bacillus thermophilus can even be used at temperatures over 90°C.

Work carried out by Smith (41) clearly illustrates that although high concentrations of sucrose protect bacterial α-amylases from thermal inactivation, their effectiveness nevertheless diminishes fairly rapidly at Brixes in excess of 50°.

The most common method of using bacterial enzymes in the South African sugar industry is to treat sirup from the penultimate vessel of the raw sirup evaporator. Although enzymatic removal of starch is not the only factor involved, there is little doubt that it has been the major contributor in reducing the starch level in South African raws from more than 450 ppm in 1957 to 110 ppm in 1973 and to the current level of less than 100 ppm.

It may be of interest to refiners to note that the use of bacterial α-amylases to reduce starch in the refinery melt has several drawbacks to its successful use. Tests at the Sugar Milling Research Institute (32) have shown that the starch in dissolved raw sugar does not respond to enzymatic hydrolysis

as readily as that in raw sirup. The reason is thought to be retrogradation of some of the starch in the solid raw sugar. A second difficulty is that the enzymes have a very low efficiency at Brixes above 60°. Our present levels of technology suggest that the logical place for starch reduction in sugar manufacture is in the raw factory.

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DISCUSSION

J. C. P. CHEN (Southdown): In South Africa, is there any work being done on breeding cane for low amylose?

M. MATIC: To my knowledge, there is no work in progress on breeding cane with low amylose content. From the physiological point of view, the cane breeders are not worried about starch; this is something they find normal. This is one reason why so little has been done up to now, plus, of course, the fact that only very recently did we find that amylose is the component of starch that is of particular importance.

J. V. LOPEZ-ONA (National Sugar): I recall that in Cuba we had at one time a starch problem, and, with the help of some cane breeding research in planting different varieties, we were able to improve the starch problem tremendously.

With regard to figure 2, about the correlation coefficient on an individual lab test basis with filterability, you did single regression analyses on each factor. If you were to run a multiple regression analysis, which you can do by variance analysis, you could break the figure down and find out the weight of each component in the correlation component.

M. MATIC: This figure was taken from the work of Yamane[1]. I believe he did just the simple regression analysis. I think if you did do the multiple regression analysis, the results would be pretty similar. The point is that the starch influence is so much greater than that of the other factors that, as long as you have considerable starch, the others are unimportant; if there were no starch present, then the importance of the other impurities would come to the fore. If one uses Murray's results, one can show that the influence of amylose on filterability is ten times as high as that of suspended matter, about four times as high as amylopectin, and twice as high as total starch. So all these other factors have a certain amount of influence on filterability, but in orders of magnitude less than amylose.

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[1]Yamane, T., Suzuki, K., and Kaga, T. 1965. Filtration-impeding materials in raw sugars of various origins. Int. Sugar J. 67: 333-337.

C. C. CHOU (Amstar): Have you found that there is preferential removal of amylose or amylopectin by press filtration?

M. MATIC: We have not looked at that at all.

EFFECT OF PHOSPHATE CONSTITUENTS ON THE SUGAR REFINING PROCESS

By Chung Chi Chou[1]

ABSTRACT

Laboratory studies were made to evaluate the effect of phosphates on the filtration and decolorization of sugar liquor. The mechanisms of phosphate interference in these processes are elucidated. The role of the phosphate constituents in the deterioration of bone chars is also discussed.

INTRODUCTION

All raw cane sugars contain a certain amount of undesirable impurities, such as gums, waxes, starch (and other polysaccharides), and proteins, in addition to phosphates and a few other inorganic salts. These occur either as colloidal material or as particles too large to be considered truly colloidal in nature. The effect of these impurities on the sugar refining process has been the subject of much investigation, both in the laboratory and the refinery.

In the present study, laboratory experiments were performed to evaluate the effect of phosphates on filtration, and also on decolorization and on the deterioration of bone char. The mechanism of phosphate interference in these processes is discussed.

PHOSPHATE AND FILTERABILITY

Filtration difficulties have been reported in the processing of certain raw sugars. A survey of the literature reveals that several classes of compounds were suspected of impeding filtration. These included starches, proteins, phosphates, silica, polysaccharides, gums, lipids and waxes. Various theories of macromolecule-ion interaction and macromolecule-filter earth interface interaction have been proposed[2]. Using the literature information, we conducted a survey of various process streams to determine the profile of non-sugar constituents in raw sugar, and to follow their behavior through the refinery. Table 1 shows the results obtained with raw sugar and washed raw sugar. Samples of other streams including washed sugar liquor, press filtered washed sugar liquor, high purity sweet water, etc. were also collected and analyzed. The linear correlation between these analytical results and the corresponding filtration test values was calculated using linear regression analysis. The results of these calculations are shown in table 2.

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[1]Manager, Operations Laboratory, Amstar Corporation, 49 South Second Street, Brooklyn, New York 11211.

[2]Napper, D. 1970. Colloid stability. Ind. Eng. Chem. Prod. Res. Develop. 9: 467-477.

TABLE 1--Non-sugar constituents in raw sugar and washed raw sugar (% on solids) (average of 16 raw sugar samples)

	Protein (%)	Starch	Poly-saccharide	Silicate	Phosphate	Ash	Alcohol insolubles
Raw sugar....	0.047	0.028	0.105	0.0032	0.0097	0.48	0.45
Washed raw sugar....	0.014	0.018	0.067	0.0020	0.0048	0.16	0.16

Analytical Results and Filterability

Analysis of the results indicates a relatively large correlation between phosphate and alcohol insolubles in washed raw sugar and the filtration values. The correlation with polysaccharides was slight, and no correlation was found between the concentration of silicate, starch, or protein in washed raw sugar and filterability.

TABLE 2--Correlation between analytical results and filterability

	r-Value	
Analytical parameter	WRS	WSL-PFWSL
Phosphate	-0.62	-0.63
Silicate	-0.15	
Starch	-0.15	-0.58
Protein	-0.08	-0.23
Alcohol insolubles	-0.54	-0.48
Polysaccharides	-0.33	-0.58

The relationship between the non-sugar constituents removed by the press filtration and the corresponding filtration values were also obtained. As is seen from table 2, the correlation between filterability and phosphate removal by press filtration is relatively large. Some correlation is also shown for the polysaccharides, starch, and the alcohol insolubles removed by press filtration.

Table 2 also shows some rather striking features:

1. While the correlation between filterability and the amount of starch present in washed raw sugar is very poor, some correlation was found with the amount of starch removed by press filtration. These results suggest that the type of starch, and not the amount of starch, affects filterability.

2. A high correlation between phosphate and alcohol insolubles, (both in the case of the absolute amount in raw sugar and the amount removed by press filtration) and the filtration values indicates that it is the amount and, perhaps, the type of phosphate and alcohol insolubles that determines filterability.

The minus signs of the r values indicate a negative correlation. This means that the filterability is inversely proportional to the concentration of the various parameters. No silicate result is reported because the silicate was found to be consistently higher after filtration, presumably due to dissolution of soluble silicate from filter earth or breakthrough of small particles of filter earth into the press filtered washed sugar liquor.

Phosphate Removal and Press Filtration

Table 3 shows the relationship between phosphate removal and press filtration. A large percentage of phosphate is removed during the press filtration. This finding is verified by the build-up of phosphate in regenerated filter aid as shown in table 4. It was thought that removal of phosphate from regenerated filter earth should result in an improvement in the filterability; this theory was confirmed by experiment. Figure 1 compares the filterability of a washed raw sugar liquor using regenerated filter earth, before and after washing with acid. The pH of the sugar liquor was adjusted with various agents and the effect of pH changes on filterability was determined. In the pH range studied, from pH 6 to pH 9, the filterability was increased by about 35% simply by acid washing the regenerated earth. The data also indicated that the use of phosphate for pH adjustment resulted in a large decrease in filterability, as expected.

PHOSPHATE ADSORPTION BY BONE CHAR

Purification of sugar liquor by bone char involves removal of organic impurities, such as anionic high molecular weight colorants, together with inorganic ash, via a "sorption" process. The spent char is then revivified for reuse. During the revivification, the organic impurities can be removed from the char by water washing and proper kilning. However, it is not economically feasible to wash off completely the inorganic ash from the char during

It can be seen from table 5 that the bulk density of new char increases from 39 lb/ft^3 to about 48 lb/ft^3 after attrition of more than one hour. Since there are practically no extremely fine particles in heavy discard char, the "fines" portion on the pan (Tyler Screen) was removed before the measurement of the bulk density of the attrited new char. The particle size and shape of the new char after attrition is presumably comparable to that of heavy discard char. The results indicate that the "shape effect" accounts for 9 lb/ft^3 increase in bulk density.

Table 6 is a summation of the analyses obtained for new and heavy discard char. To simplify the interpretation of the analytical data, the analyses were made on the "ash" content of the chars and the results were calculated back to the sample on an "as is" basis.

The amounts of each constituent retained by the heavy discard char per cubic foot are also listed in table 6. The calculations are based on the bulk density of the attrited new char of 48 lb/ft^3 and that of discard char of 67 lb/ft^3. The data reveal a striking feature: The "adsorbed" tricalcium phosphate is the main factor responsible for the increase in bulk density of the char. The data also indicate that discard char retained 17.8 lb/ft^3 of tricalcium phosphate compared to 1.2 lb/ft^3 of calcium sulfate retained.

TABLE 6--Analysis of a new and a heavy discard bone char

	$CaSO_4$	SiO_2	$Ca_3(PO_4)_2$	Bulk density (lb/ft^3)
New char	0.28%	1.2%	69.7%	39
Heavy discard	2.0%	2.7%	76.5%	67
Amount increased or retained for discard (lb/ft^3)	1.2	1.3	17.8	28

Two explanations may be proposed to account for the great tendency of the chars to retain the calcium phosphate salt. First, bone char is about 80% calcium phosphate, in hydroxyapatite form, which may serve as the nucleus for the crystallization of calcium phosphate salt from sugar liquor. Second, the solubility of calcium phosphate is extremely low in aqueous solution, and it is conceivable that, once calcium phosphate from sugar liquor is incorporated into the crystal structure of hydroxyapatite in the bone char, it is resistant to being washed out in the char washing process.

This postulation in terms of the autocatalytic effect and solubility of the hydroxyapatite in bone char is in agreement with the findings of A. S. Posner as presented in his recent publication, "Conversion of Amorphous Calcium Phosphate to Microcrystalline Hydroxyapatite"[5].

To check further on the "sorption" of tricalcium phosphate by bone char, an affination sirup was recirculated through a char column for two days. The ash and phosphorus pentoxide content of the affination sirup before and after char filtration was analyzed and is shown in table 7. The data indicate that 29.4% of P_2O_5 was adsorbed and 19.1% of total ash was removed from affination sirup by the char during the recycle operation. Similar results were obtained using washed raw sugar liquor, as shown in table 8. The phosphate is somewhat preferentially "sorbed," probably because the hydroxyapatite of the char served as the nucleus for the crystallization of calcium phosphate salt, as previously described. Although the mechanism of the "sorption" of tricalcium phosphate has not yet been studied, the results do show that the phosphate salt is removed by the char. Since the retention time of bone char in the char house may be up to two years, and since the char may undergo two hundred cycles before discard due to high bulk density, it is conceivable that each cubic foot of discard char may accumulate 17 lbs of phosphate salt during its usage in the refinery, as calculated from the experimental data.

TABLE 7--Adsorption of $Ca_3(PO_4)_2$ by new char from affination sirup

	Before char filtration	After char filtration	% Retained by char
% Ash	1.88	1.51	19.1
% P_2O_5 on ash	1.36	0.96	29.4

TABLE 8--Phosphate "sorbed" by the stock chars during laboratory batch decolorization of washed raw sugar liquor

Char sample	Phosphate (ppm)		% Phosphate removal
	Control liquor	After decolorization	
Stock char A	35.7	13.8	61.3
Stock char B	35.0	14.4	58.9

[5]Boskey, A. L., and Posner, A. S. 1973. Conversion of amorphous calcium phosphate to microcrystalline hydroxyapatite. A pH-dependent, solution-mediated, solid-solid conversion. J. Phys. Chem. 77: 2313-2315.

EFFECT OF PHOSPHATE "SORPTION" ON DH AND DECOLORIZATION OF CHAR

"DH" of char is a measurement of the concentration of Ca + Mg in the water extract of char expressed in millinormal units. Due to the extremely low solubility of calcium phosphate salts, the adsorption of phosphate from sugar liquor will be enhanced by the presence of Ca + Mg in the char. We have made a study of the relationship between the phosphate adsorption, DH and decolorization performance of char. The char was treated with water and sodium phosphate solution at various ratios and concentrations. The length of mixing time ranged from one to two hours. The phosphate remaining in the solution after mixing was analyzed, and the DH and color-removal capacity of the treated chars were determined. The results are summarized in table 9.

TABLE 9—Effect of phosphate adsorption on DH and decolorization of char

Run No.	Char treatment	P_2O_5 (ppm) remaining in solution after mixing	DH of treated char	% Decolorization
1	Char "as is" (not treated)	---	6.4	69.7
2	Char:water = 1:1 1 h mixing	0.2	6.1	69.9
3	Char:water = 1:2 2 h mixing	1.0	4.6	69.9
4	Char:Na_2HPO_4 solution (1600 ppm) = 1:1 1 h mixing	0.2	3.9	69.6
5	Char:Na_2HPO_4 solution (3200 ppm) = 1:1 1 h mixing	1.2	2.2	63.8
6	Char:Na_2HPO_4 solution (6400 ppm) = 1:1 2 h mixing	1.2	0.24	45.4

It is seen that nearly all the phosphate was "sorbed" by the char as expected. For the char studied, the phosphate "sorption" was up to 0.6% based on the weight of the char. Table 9 also shows that the DH of the char treated with Na_2HPO_4 solution was reduced drastically. In general, the DH of

the treated char is inversely proportional to the concentration of Na_2HPO_4 solution used. The explanation is simply the precipitation of phosphate ions with the available Ca + Mg in the char.

The relationship between DH and decolorization of char is interesting. At DH values of 6.4 to 2.2, the percent decolorization is not greatly affected. As the DH decreases from 2.2 to 0.24 the percent color removal drops from 64% to 45%. This large decrease in decolorization at low DH is probably due to the deficiency of calcium ions in the system. The observation indicates the importance of maintaining an optimum calcium ion level in liquor over char in order to facilitate decolorization. This phenomenon can be readily explained in terms of the mechanism of decolorization.

The adsorbent-adsorbate interaction responsible for decolorization can be classified due to both ion exchange and adsorption. In the ion exchange reactions, ionic colorants are chemically retained by adsorbents due to exchange of ions of similar charge. An example is the removal of anionic colorant by anion exchanger. In the adsorption process, nonionic colorants are retained by adsorbents through specific and/or nonspecific interaction.

Molecules possessing functional groups of the type OH or NH, and capable of forming hydrogen bonding, are adsorbed through specific interaction. This type of bonding is characterized by relatively high heat of adsorption. An example is the bonding of the group of basic colorants to the hydroxyl group of a phenolformaldehyde resin. In contrast to hydrogen bonding, hydrophobic bonding is related to the nonspecific adsorption of molecules on the adsorbent surface. The heat of adsorption is low and determined mainly by the dispersion forces. An example of this type is the bonding of the hydrophobic portion of colorants to that of adsorbents.

As a rule of thumb for adsorption, the hydrophobic or nonpolar molecules are attracted to hydrophobic or nonpolar surfaces, while hydrophilic or polar molecules are attracted to hydrophilic or polar surfaces.

Since bone char consists of an aromatic matrix structure with a large hydrophobic surface, it is conceivable that colorant removal from sugar liquor by carbonaceous adsorbent is, to a large extent, of a nonspecific type of adsorption in nature.

From the above discussion, it is evident that the observed high degree of decolorization by bone char is expected when a certain level of excess calcium ions is present in the system. The anionic colorant can react with calcium ion to form nonionic colorant. The conversion of ionic to nonionic colorant will result in an increase in the hydrophobic nature of the colorant and thus an increase in the decolorization. The pairing of calcium ion and ionic colorant also facilitates the decolorization, due to the fact that the solubility of the colorant is reduced considerably by the formation of nonionic calcium salts of the colorant. In general, the adsorption of ionic species, including ionic colorants, from aqueous solution to a hydrophobic surface is less likely because of the high degree of hydration of the ionic center by the water molecules.

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TABLE 3--Phosphate removal by press filtration

Sample	% Phosphate X 1000
Washed raw sugar liquor (from melter). Average of 5 samples	5.3
Washed raw sugar liquor (from press feed). Average of 7 samples	14.6
Press filtered washed sugar liquor. Average of 8 samples	0.36
Raw sugar	15.5
Washed raw sugar	5.0

TABLE 4--Ash and P_2O_5 content of various materials

	Raw sugar A	Refined sugar raw sugar A origin	Blackstrap	New filter aid	Regenerated filter aid
% Thermal ash	0.35	0.007	11.4	---	---
% P_2O_5 on ash	4.2	3.9	1.6	0.4	1.75

each washing cycle. The resulting gradual accumulation of inorganic ash on the char is, to a large extent, responsible for its increase in bulk density with time in service. We are interested in a study of what constituents are most retained on bone char and are responsible for the increase in bulk density of char.

It is widely known that the bulk density of an adsorbent depends, to a certain extent, on the interparticle configuration inside the container. Since the new char particles have more "edges" than those of the heavy discard

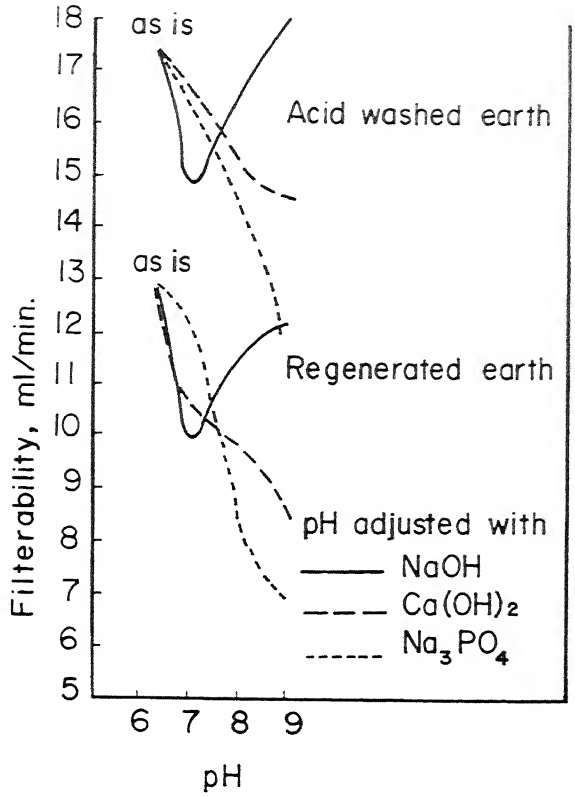

FIGURE 1--Effect of acid washing of regenerated earth on filterability.

char, it is believed that the "shape effect" of the new char particles is partially responsible for the increase in bulk density as char undergoes service. To confirm this point of view, attrition tests were carried out on the new char for various lengths of time and the results are shown in table 5. The attrition test, according to the Bone Char Research Project Reports[4], simulates the effect of service on the size and shape of bone chars, due to mechanical abrasion which tends to round off the edges of the chars.

TABLE 5--Effect of attrition time on the bulk density of new char (lb/ft^3)

	As is	1 h	2 h	3 h
Bulk density	39.5	47	48.4	48.7

[4]Carpenter, F. G. 1957. Development of a new test for the abrasion hardness of bone char. Proc. Tech. Sess. Bone Char 5: 99-135.

It can be seen from table 5 that the bulk density of new char increases from 39 lb/ft^3 to about 48 lb/ft^3 after attrition of more than one hour. Since there are practically no extremely fine particles in heavy discard char, the "fines" portion on the pan (Tyler Screen) was removed before the measurement of the bulk density of the attrited new char. The particle size and shape of the new char after attrition is presumably comparable to that of heavy discard char. The results indicate that the "shape effect" accounts for 9 lb/ft^3 increase in bulk density.

Table 6 is a summation of the analyses obtained for new and heavy discard char. To simplify the interpretation of the analytical data, the analyses were made on the "ash" content of the chars and the results were calculated back to the sample on an "as is" basis.

The amounts of each constituent retained by the heavy discard char per cubic foot are also listed in table 6. The calculations are based on the bulk density of the attrited new char of 48 lb/ft^3 and that of discard char of 67 lb/ft^3. The data reveal a striking feature: The "adsorbed" tricalcium phosphate is the main factor responsible for the increase in bulk density of the char. The data also indicate that discard char retained 17.8 lb/ft^3 of tricalcium phosphate compared to 1.2 lb/ft^3 of calcium sulfate retained.

TABLE 6--Analysis of a new and a heavy discard bone char

	$CaSO_4$	SiO_2	$Ca_3(PO_4)_2$	Bulk density (lb/ft^3)
New char	0.28%	1.2%	69.7%	39
Heavy discard	2.0%	2.7%	76.5%	67
Amount increased or retained for discard (lb/ft^3)	1.2	1.3	17.8	28

Two explanations may be proposed to account for the great tendency of the chars to retain the calcium phosphate salt. First, bone char is about 80% calcium phosphate, in hydroxyapatite form, which may serve as the nucleus for the crystallization of calcium phosphate salt from sugar liquor. Second, the solubility of calcium phosphate is extremely low in aqueous solution, and it is conceivable that, once calcium phosphate from sugar liquor is incorporated into the crystal structure of hydroxyapatite in the bone char, it is resistant to being washed out in the char washing process.

This postulation in terms of the autocatalytic effect and solubility of the hydroxyapatite in bone char is in agreement with the findings of A. S. Posner as presented in his recent publication, "Conversion of Amorphous Calcium Phosphate to Microcrystalline Hydroxyapatite"[5].

To check further on the "sorption" of tricalcium phosphate by bone char, an affination sirup was recirculated through a char column for two days. The ash and phosphorus pentoxide content of the affination sirup before and after char filtration was analyzed and is shown in table 7. The data indicate that 29.4% of P_2O_5 was adsorbed and 19.1% of total ash was removed from affination sirup by the char during the recycle operation. Similar results were obtained using washed raw sugar liquor, as shown in table 8. The phosphate is somewhat preferentially "sorbed," probably because the hydroxyapatite of the char served as the nucleus for the crystallization of calcium phosphate salt, as previously described. Although the mechanism of the "sorption" of tricalcium phosphate has not yet been studied, the results do show that the phosphate salt is removed by the char. Since the retention time of bone char in the char house may be up to two years, and since the char may undergo two hundred cycles before discard due to high bulk density, it is conceivable that each cubic foot of discard char may accumulate 17 lbs of phosphate salt during its usage in the refinery, as calculated from the experimental data.

TABLE 7--Adsorption of $Ca_3(PO_4)_2$ by new char from affination sirup

	Before char filtration	After char filtration	% Retained by char
% Ash	1.88	1.51	19.1
% P_2O_5 on ash	1.36	0.96	29.4

TABLE 8--Phosphate "sorbed" by the stock chars during laboratory batch decolorization of washed raw sugar liquor

Char sample	Phosphate (ppm)		% Phosphate removal
	Control liquor	After decolorization	
Stock char A	35.7	13.8	61.3
Stock char B	35.0	14.4	58.9

[5]Boskey, A. L., and Posner, A. S. 1973. Conversion of amorphous calcium phosphate to microcrystalline hydroxyapatite. A pH-dependent, solution-mediated, solid-solid conversion. J. Phys. Chem. 77: 2313-2315.

EFFECT OF PHOSPHATE "SORPTION" ON DH AND DECOLORIZATION OF CHAR

"DH" of char is a measurement of the concentration of Ca + Mg in the water extract of char expressed in millinormal units. Due to the extremely low solubility of calcium phosphate salts, the adsorption of phosphate from sugar liquor will be enhanced by the presence of Ca + Mg in the char. We have made a study of the relationship between the phosphate adsorption, DH and decolorization performance of char. The char was treated with water and sodium phosphate solution at various ratios and concentrations. The length of mixing time ranged from one to two hours. The phosphate remaining in the solution after mixing was analyzed, and the DH and color-removal capacity of the treated chars were determined. The results are summarized in table 9.

TABLE 9—Effect of phosphate adsorption on DH and decolorization of char

Run No.	Char treatment	P_2O_5 (ppm) remaining in solution after mixing	DH of treated char	% Decolorization
1	Char "as is" (not treated)	---	6.4	69.7
2	Char:water = 1:1 1 h mixing	0.2	6.1	69.9
3	Char:water = 1:2 2 h mixing	1.0	4.6	69.9
4	Char:Na_2HPO_4 solution (1600 ppm) = 1:1 1 h mixing	0.2	3.9	69.6
5	Char:Na_2HPO_4 solution (3200 ppm) = 1:1 1 h mixing	1.2	2.2	63.8
6	Char:Na_2HPO_4 solution (6400 ppm) = 1:1 2 h mixing	1.2	0.24	45.4

It is seen that nearly all the phosphate was "sorbed" by the char as expected. For the char studied, the phosphate "sorption" was up to 0.6% based on the weight of the char. Table 9 also shows that the DH of the char treated with Na_2HPO_4 solution was reduced drastically. In general, the DH of

the treated char is inversely proportional to the concentration of Na_2HPO_4 solution used. The explanation is simply the precipitation of phosphate ions with the available Ca + Mg in the char.

The relationship between DH and decolorization of char is interesting. At DH values of 6.4 to 2.2, the percent decolorization is not greatly affected. As the DH decreases from 2.2 to 0.24 the percent color removal drops from 64% to 45%. This large decrease in decolorization at low DH is probably due to the deficiency of calcium ions in the system. The observation indicates the importance of maintaining an optimum calcium ion level in liquor over char in order to facilitate decolorization. This phenomenon can be readily explained in terms of the mechanism of decolorization.

The adsorbent-adsorbate interaction responsible for decolorization can be classified due to both ion exchange and adsorption. In the ion exchange reactions, ionic colorants are chemically retained by adsorbents due to exchange of ions of similar charge. An example is the removal of anionic colorant by anion exchanger. In the adsorption process, nonionic colorants are retained by adsorbents through specific and/or nonspecific interaction.

Molecules possessing functional groups of the type OH or NH, and capable of forming hydrogen bonding, are adsorbed through specific interaction. This type of bonding is characterized by relatively high heat of adsorption. An example is the bonding of the group of basic colorants to the hydroxyl group of a phenolformaldehyde resin. In contrast to hydrogen bonding, hydrophobic bonding is related to the nonspecific adsorption of molecules on the adsorbent surface. The heat of adsorption is low and determined mainly by the dispersion forces. An example of this type is the bonding of the hydrophobic portion of colorants to that of adsorbents.

As a rule of thumb for adsorption, the hydrophobic or nonpolar molecules are attracted to hydrophobic or nonpolar surfaces, while hydrophilic or polar molecules are attracted to hydrophilic or polar surfaces.

Since bone char consists of an aromatic matrix structure with a large hydrophobic surface, it is conceivable that colorant removal from sugar liquor by carbonaceous adsorbent is, to a large extent, of a nonspecific type of adsorption in nature.

From the above discussion, it is evident that the observed high degree of decolorization by bone char is expected when a certain level of excess calcium ions is present in the system. The anionic colorant can react with calcium ion to form nonionic colorant. The conversion of ionic to nonionic colorant will result in an increase in the hydrophobic nature of the colorant and thus an increase in the decolorization. The pairing of calcium ion and ionic colorant also facilitates the decolorization, due to the fact that the solubility of the colorant is reduced considerably by the formation of nonionic calcium salts of the colorant. In general, the adsorption of ionic species, including ionic colorants, from aqueous solution to a hydrophobic surface is less likely because of the high degree of hydration of the ionic center by the water molecules.

It should be pointed out that the conversion of anionic colorant to an electrically neutral molecule can also be achieved by increasing the hydronium ion concentration by lowering the pH of the sugar liquor. This also accounts for the general observation that the percent color removal increases as the pH of the liquor decreases.

CONCLUSIONS

The filterability of sugar liquor is inversely related to the amount of phosphate in the sugar liquor and to the amount removed by press filtration.

"Sorption" of phosphate by bone char accelerates the increase in the bulk density of the char.

"Sorption" of phosphate by bone char is facilitated by the high DH of the char.

Phosphate ion competes with anionic colorant for calcium ion in the decolorization process. High phosphate ion concentration in sugar liquor will preferentially precipitate with calcium ion on hydroxyapatite result in calcium deficiency in the system and poor decolorization.

Effect of calcium ion on decolorization may be explained in terms of the hydrophilic-hydrophobic balance of the colorant and the nature of adsorbent.

It is obvious that the phosphate content of sugar liquor should be reduced in order to extend the life of char and to improve decolorization.

COMPOSITION OF ACID BEVERAGE FLOC

By E. J. Roberts[1] and F. G. Carpenter[2]

(Presented by E. J. Roberts)

ABSTRACT

Acid beverage floc has been isolated from one sample of refined cane sugar and its composition has been studied. The major inorganic component was found to be silicon dioxide. The organic portion of the isolated floc consisted of polysaccharides and protein. The component sugars of the polysaccharides and the component amino acids were identified and quantitatively estimated.

INTRODUCTION

Refined sucrose from sugarcane or sugar beets is one of the purest commercial chemicals available. In spite of its high purity, sucrose sometimes contains a few parts per million of material which precipitates when its solution is acidified. The precipitate is usually colloidal and invisible at first, but upon standing gradually coagulates to form a voluminous precipitate called floc which slowly settles to the bottom of the container. Floc has been a source of considerable trouble to sugar refiners as it is objectionable in soft drinks and acidic pharmaceutical sirups.

It was found, several years ago, that the floc formed in beet sugar solutions was composed chiefly of a saponin and its derivatives (5, 12)[3]. That problem was solved by modifications and improvements of the processing methods.

Unfortunately the composition of floc in cane sugar was found to be more complex. However, improvements in processing methods over the years have decreased the incidence of floc formation considerably.

Only two papers have appeared in the literature on the subject of sugarcane floc: one by Stansbury and Hoffpauir (11), and the other by Cohen, Dionisio, and Drescher (2).

Stansbury and Hoffpauir (11) studied five different floc-forming sugars and found that the isolated floc consisted of starch, lipids or waxes, protein, silica, and decolorizing carbon. They reported that the sugars containing the largest amount of decolorizing carbon gave the largest amount of floc and

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found that no other constituents present in the floc gave such significant correlation.

Cohen et al. (2) studied the alcohol floc (6) (haze formed by addition of alcohol to aqueous solution of sugar) and acid floc formation and the composition of the floc obtained from 28 samples of refined sugar. They found that all sugars that were acid floc positive were also alcohol floc positive but that the reverse was not necessarily true. No sugar was found that was acid floc positive and alcohol floc negative. They found no correlation between the amount of decolorizing carbon and the amount of floc formed as reported by Stansbury and Hoffpauir; however, they did find a high correlation between the amount of protein present in the sugar and the amount of floc formed. The addition of protein to a solution of acid floc negative, alcohol floc positive sugar made it acid floc positive. Since the chemical composition of the alcohol floc was found to be practically the same as that of the acid floc, except for the absence of protein in the alcohol floc, Cohen et al. concluded that the key ingredient in acid floc formation was protein.

In neither of these papers was a detailed determination made of the composition of the organic portion of the floc. The purpose of the current study was to determine the composition of cane sugar floc with special emphasis on the organic portion. The first essential for the study was a substantial quantity of a sugar that formed beverage floc readily. However, only one sample of floc forming sugar was found, in two years of testing. This is a mark of recommendation for current processing by the sponsoring refineries, but means that any conclusions drawn from this work can only apply to the one sugar sample employed.

It should be pointed out that the analyses reported in this paper were made on several different floc preparations which may differ from one preparation to another even though they are all from the same lot of sugar.

MATERIALS AND METHODS

Isolation of Floc

Sirups were prepared by the Coca-Cola Co. floc test (5) procedure for the large scale isolation of floc. Sucrose, 10.8 kg, was dissolved in 9.5 l water and 500 ml 37% formaldehyde in a glass container. The solution was approximately 54 Brix. The pH was adjusted to 1.5 with 85% phosphoric acid. The solution was allowed to stand at room temperature (about 25° C). On the fourth day floc had begun to form and after 10 days it had settled to the bottom of the container. The supernatant liquid was siphoned off and the sediment separated from the remaining solution by batch centrifugation at 28,000 g. All the sediment was transferred to one centrifuge tube, suspended in 70% ethanol and recentrifuged. This washing procedure was repeated twice more to remove all the sugar. The sediment was finally suspended in about 15 ml of water and freeze dried. The weight of the dried floc was 0.118 g (0.001092% of total sugar, or 10.92 ppm). The average yield of floc from 4 preparations was approximately 10 ppm.

Analysis of Floc

Inorganic Constituents

The floc was analyzed for nitrogen, phosphorus, silicon dioxide, and ash by a commercial analytical laboratory.

Starch

Starch was determined by the iodine colorimetric method of Balch (1).

Hydrolysis of Floc Polysaccharides

An amount of 0.41 g of dry floc was hydrolyzed under mild acid conditions by refluxing in 100 ml of 2N sulfuric acid for 4 h. The solution was cooled and centrifuged to separate the insoluble material. The sediment was washed with two 50 ml portions of water in the centrifuge tube. The insoluble material was suspended in 15 ml of water and freeze dried. It weighed 0.28 g, 68.24% of the total floc. The supernatant solution from the first centrifugation and the washings were combined and sulfuric acid was removed by the addition of a slight excess of solid barium hydroxide. The excess barium ions were removed by bubbling carbon dioxide through the solution until neutral. The barium salts were removed by filtration on a filter-aid mat. The filtrate was concentrated on a rotary evaporator below 60° C, to about 15 ml and freeze dried. The dry hydrolyzate weighed 0.125 g, 30.6% of the total floc.

Identification and Determination of Sugars in Hydrolyzed Floc Polysaccharides

The component sugars in the hydrolyzed floc polysaccharides were identified by comparison of retention times and peak enhancement with known sugars on gas-liquid chromatography. The gas chromatograph was a Hewlett-Packard model 5750 equipped with a flame ionization detector. The column was a 1/8" OD stainless steel tube, 6 ft long packed with 10% OV-1 on Chromosorb HP, 80-100 mesh. The column was operated isothermally at 180° C with a carrier gas flow rate of 17 ml per min. The sugars were silylated with trimethylchlorosilane and hexamethyldisilazane in pyridine prior to gas chromatography. However, since some of the peaks overlapped it was necessary first to establish the anomer ratio of each sugar in pyridine so that the areas of hidden peaks could be calculated. Sorbitol was used as the internal standard in the quantitative estimation of the sugars.

Hydrolysis of Floc Proteins

The protein in the floc was hydrolyzed under more strenuous conditions than the polysaccharides, by refluxing 0.235 g of dry floc in 100 ml of 6N hydrochloric acid for 16 h (4). The solution was filtered through a mat of filter aid and the mat was washed with 100 ml of water. The combined filtrate and washings was concentrated on a rotary evaporator below 60° C to about 25 ml. Another 100 ml of water was added and the solution again evaporated to

about 25 ml. This procedure was repeated three more times to remove most of the hydrochloric acid. The solution was finally evaporated to about 2 ml and freeze dried.

Identification and Determination of Amino Acids in Hydrolyzed Floc Protein

The component amino acids in the hydrolyzed floc protein were identified by comparison of retention times and peak enhancement with known amino acids on gas-liquid chromatography, and confirmed by thin layer chromatography using butanol:acetic acid:water 4:1:1 as the developing solvent. The gas chromatograph was a Packard model 7300 equipped with a flame ionization detector. The column was 4 mm ID glass tube, 2 ft long, packed with an experimental packing obtained from Perkin-Elmer, the composition of which has not been divulged. The injection port was equipped with a glass insert and the column was programmed to go from 100° C to 250° C at 90°/min. The amino acids were converted to the n-propyl-N-acetyl derivatives before chromatography (3). Tranexamic acid was used as an internal standard for quantitative estimation of the amino acids (trans-4-(aminomethyl)cyclohexanecarboxylic acid)."

Determination of Fats, Waxes and Lipids

The fats, waxes, and lipids were determined by extracting 0.247 g of dry floc with ten 10 ml portions of chloroform on a millipore filter (0.45μ) (7). The combined chloroform extracts were evaporated to about 1 ml, transferred to a tared 2 ml vial and evaporated to dryness. The residue weighed 0.013 g, or 5.26% of the floc.

Effect of Temperature on Floc Formation

Five l of 54 Brix sirup was prepared; the pH was adjusted to 1.5 with phosphoric acid. The solution was divided into five 1 l portions; one portion was stored at each of the following temperatures and examined each day for floc formation: 4° C, 25° C, 40° C, 60° C, and 80° C.

Effect of pH on Floc Formation

Eight 1 l batches of 54 Brix solutions were prepared and adjusted to pH 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 with phosphoric acid or sodium hydroxide. The solutions were stored at 25° C (room temperature) and examined each day for 10 days for floc formation.

RESULTS

The average yield of dried floc from this sugar was 10 ppm. A gross analysis of the floc is shown in table 1. The silicon dioxide content of this sample is considerably higher than that reported by Stansbury and Hoffpauir (11), but the starch, fats, waxes, and lipids are much lower than reported earlier.

TABLE 1--Gross analysis of floc

Analysis	Percent of floc
Dry ash	69.80
Silicon dioxide	63.50
Nitrogen	0.63
Phosphorus	0.57
Fats, waxes and lipids	5.26
Starch	5.50

Floc Polysaccharides

Results of hydrolysis of the floc polysaccharides in 2N sulfuric acid are shown in table 2. It may be seen that the fraction of the floc which was insoluble in refluxing 2N sulfuric acid amounted to 68.24%. This insoluble fraction was found to be 75.00% silicon dioxide.

TABLE 2--Hydrolysis of floc polysaccharides, by refluxing in 2N sulfuric acid for 4 hours

Soluble portion	30.60%
Insoluble portion	68.24%

The sugars derived from hydrolysis of the floc polysaccharides were identified by comparison of retention times and peak enhancement with pure known sugars. The chromatogram is reproduced in figure 1.

The component sugars were identified as arabinose, rhamnose, xylose, mannose, fructose, galactose, and glucose. An examination of this chromatogram shows that some of the peaks overlap; however, since one peak of each sugar was clear, it was possible to make quantitative estimations of the sugars, as described above.

The quantitative analysis of the hydrolyzed floc polysaccharides is shown in table 3. In a study of the polysaccharides in sugarcane, presented at the

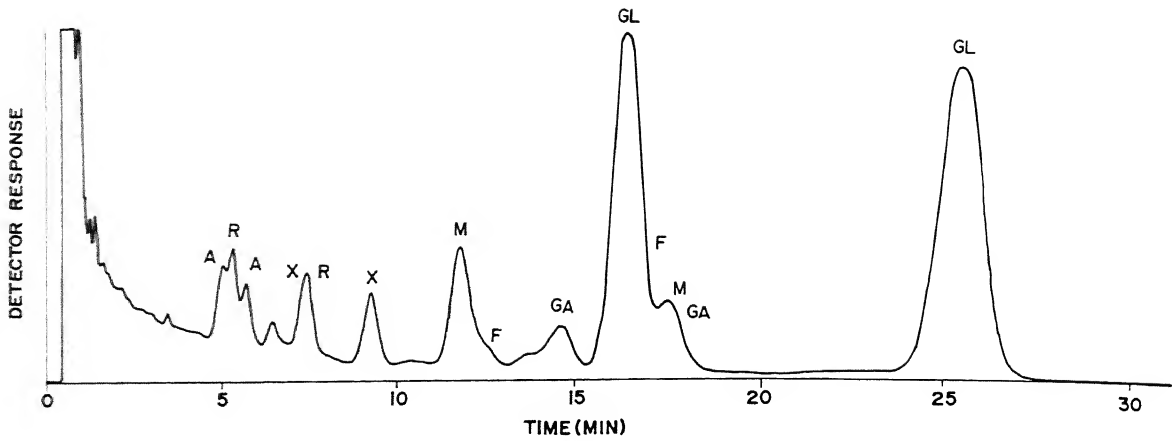

FIGURE 1--Chromatogram of floc polysaccharides hydrolysis products.

A = Arabinose
R = Rhamnose
X = Xylose
M = Mannose
F = Fructose
GA = Galactose
GL = Glucose

TABLE 3--Estimation of sugars in hydrolyzed floc polysaccharides

Sugar	Percent of floc
Arabinose	0.63
Rhamnose	0.48
Xylose	0.69
Mannose	1.21
Galactose	0.58
Glucose	14.10
Total	17.70%

1964 Technical Session on Cane Sugar Refining, Roberts, Jackson and Vance (8) reported the identification of these same sugars in polysaccharides from

molasses and other sugarcane products. Glucose was the predominant sugar in these polysaccharides as it is in the floc polysaccharide. However, in unpublished work the same workers found that the polysaccharide from freshly cut cane contained very little glucose and much larger quantities of the other sugars. The other sugars were considered to be in the indigenous polysaccharides which are present in all cane, while the glucose is derived mostly from dextran formed by microorganisms during the interval between harvesting and crushing.

Floc Proteins

The amino acids identified and estimated in the hydrolyzed floc protein are shown in table 4.

TABLE 4--Identification and estimation of amino acids in hydrolyzed protein

Amino acid	Percent of floc
Aspartic acid	0.14
Alanine	0.11
Leucine	0.10
Glutamic acid	0.09
Serine	0.09
Threonine	0.09
Valine	0.09
Glycine	0.09
Isoleucine	0.06
Phenylalanine	0.05
Proline	0.04
Arginine	<0.01
Lysine	<0.01
Tyrosine	<0.01
Histidine	<0.01

The quantities of amino acids are in about the same order of abundance as was found in cane juice (9) except that glutamic acid content was found to be lower. The protein contained in the floc was not precipitated by the usual protein precipitants after dispersion. This behavior indicates that the protein was possibly fragmented during some stage of sugar processing. This could account for the lower proportion of glutamic acid in the floc protein than was found in the cane juice protein.

Temperature, pH, and Floc

The effect of temperature on floc formation is shown in table 5.

TABLE 5--Effect of temperature on floc formation

Temperature (°C)	Days (+ = floc, - = no floc)
	1,2,3,4,5,6,7,8
4	- - - - - - - +
25	- - - +
40	- +[1]
60	- +[2]
80	- +[3]

[1]The floc settled much faster than at lower temperatures.

[2]The solution developed some color after 2 days.

[3]The solution developed considerable color after 2 days.

The floc required 8 days to form at 4°C and settled extremely slowly. It was visible on the fourth day at 25°C (room temperature), and settled at a moderate rate. At 40°C, 60°C, and 80°C the floc was visible on the second day and settled very quickly, possibly because of the decreased viscosity of the solution. At 60°C and 80°C considerable color developed in the solution after 2 or 3 days.

The effect of pH on floc formation is shown in table 6. The floc formed more slowly at pH 1 than at slightly higher pH values. The time required was longer at pH values above 4.0; no floc was visible in the solution at pH 6.0 and 7.0 after 10 days.

TABLE 6—Effect of pH on floc formation (pH before adjustment = 5.8)

pH	Days (+ = floc, - = no floc) 1,2,3,4,5,6,7,8,9,10
1.0	- - - - +
1.5	- - - +
2.0	- - - +
3.0	- - - +
4.0	- - - +
5.0	- - - - - - - - +
6.0	- - - - - - - - - -
7.0	- - - - - - - - - -

SUMMARY

Acid beverage floc has been isolated from one sample of refined sugar. Analysis of the floc showed it to be composed of silicon dioxide, polysaccharides, protein, and fats, waxes, and lipids, with silicon dioxide as the chief component. Analysis of the hydrolyzed polysaccharides showed the presence of arabinose, rhamnose, xylose, mannose, galactose, and glucose. The amino acids identified in the hydrolyzed protein were aspartic acid, glutamic acid, leucine, isoleucine, valine, threonine, glycine, serine, proline, arginine, lysine, tyrosine, and histidine.

Obviously no general analytical conclusions can be drawn from a single sample of floc forming sugar. Several more samples of floc forming sugar will be required before such conclusions can be attempted.

ACKNOWLEDGMENT

The authors are indebted to Mrs. J. H. Conkerton of this Research Center for quantitative estimations of the amino acids.

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DISCUSSION

J. F. DOWLING (CPC International): What was the origin of the raw sugar that caused that floc?

E. J. ROBERTS: That was a Phillipines raw.

J. F. DOWLING: I think everybody is familiar with the floc problems with the Australian raw from Port Mackay. What Earl needs now are samples of sugar from different origins that give floc. They don't have to be heavy flocculating sugars--but a variety of raw cane sugars from around the world is required to give a decent profile.

W. R. TUSON (Colonial Sugars): A question that is important to me is, are we talking about this problem in the singular, when we should be using the plural. We have various definitions, like alcohol insoluble floc, and various tests, like the Coca-Cola tests. Are we talking about one reaction or a combination of factors and reactions? Do you have any feeling at this point about this?

E. J. ROBERTS: At this time we have only had one flocculating sugar, and it would be hard to answer your question on that basis. If I were to make a guess, I would say that there probably is more than one kind of acid floc. There may be one factor that causes floc all the time, or there may be more than one. Since we had only one sample of flocculating sugar, it is impossible to answer that question definitely.

F. G. CARPENTER (C.S.R.R.P.): Did you find that same polysaccharide in any other samples of sugar?

E. J. ROBERTS: The only other sample of floc we have was isolated by Stansbury some 15 years ago, at the Southern Regional Research Center[1]; he had a small amount of it left and gave it to me. I hydrolyzed it, and we ran the hydrolyzate on the gas chromatograph, and we found exactly the same pattern of sugars as shown in figure 1.

M. MATIC (Sugar Milling Research Institute): On your analysis of the floc (table 1) you showed it to be about 70% inorganic, and the organic part seemed to be a minor constituent. Do you think that this minor organic part is responsible for the floc formation?

E. J. ROBERTS: We think it may be a combination of both the organic and inorganic parts.

J. F. DOWLING: I think that if you had a lot of starch--and at one time, years ago, we did blame starch for the floc problem--it would make the floc appear bigger. If I had a theory, it would be a two factor X-Y theory, where you need a minor constituent for nucleation, plus another constituent--perhaps the combination of a polysaccharide and a silica. When you form a nucleus, the starch goes along into it, as does the activated carbon. A dirty sugar increases the problem--no question about that. The thing that keys nucleation is very hard to pinpoint: whether it is a polysaccharide or not, I'm not sure. We've isolated some material and made up a batch with it with no sugar, and we've caused floc. What we are looking for is a model system, where we can pull the material X out of a sugar, put it into water and acid, but with no sugar, and see if it causes floc.

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[1] Stansbury, M. F. and Hoffpauir, C. L. 1959. Composition of "floc" formed in acidified sirups from refined granulated cane sugar. J. Agric. and Food Chem. 7: 353-358.

E. J. ROBERTS: We still have to find out whether this polysaccharide, when added back to a nonfloccing sugar, will cause floc.

J. F. DOWLING: Another curious circumstance occurred with a very bad floccing sugar (from Port Mackay) which we saved for over 5 years. We just recently tried again to make floc with this sugar, and it would not floc.

F. G. CARPENTER: Many things can be added to sugar that will cause a floc. For instance, some of the polyphenolic plant pigments which we have identified were noted to be floc forming, and when we added these to sugar, a floc was formed. This is not necessarily the acid beverage floc. Adding something to a sugar and causing a floc does not prove that the additive is the substance which causes the acid floc.

S. E. GEORGE (B. C. Sugar Refining Co.): At B. C. we get a lot of Australian raws, and we have no trouble with floc. Perhaps this is because we have cooperative customers. We continually check for floc, but have found none recently.

J. F. DOWLING: Undoubtedly the worse floccing sugar is that from Port Mackay in Australia, although it is excellent looking sugar, and refines well. We are sure that many other people have also experienced floc problems with sugar from that port. We are certainly not, however, condemning all Australian raw sugar.

K. R. HANSON (Amstar): Have you run any infrared spectra on the isolated flocs? We think that would be helpful.

E. J. ROBERTS: No, not yet. There is such a mixture of components that it would be difficult to get a very meaningful spectrum.

K. R. HANSON: We think there may be one crucial floc factor that does show up on infrared.

OPTIMUM CONDITIONS FOR DETERMINING INDIVIDUAL MINOR CONSTITUENTS IN CANE SUGAR BY GAS-LIQUID CHROMATOGRAPHY

By Mary An Godshall and Earl J. Roberts[1]

(Presented by Mary An Godshall)

ABSTRACT

In measuring the minor constituents in commercial sugars, it is necessary first to separate the constituents from the large bulk of sucrose. This is best done by liquid-liquid extraction from a solution of the sugar. The optimum extraction conditions and subsequent gas-liquid chromatography conditions for several compounds have been evaluated and the resulting precision of the entire determination evaluated.

INTRODUCTION

In working with the minor constituents present in commercial cane sugar, the investigator is faced with the extremely difficult task of separating the minute quantity of constituents from the sucrose. Many of these compounds are very similar to sucrose in solubility behavior, and are often incorporated into the sucrose crystal as an impurity rather than adsorbed onto the surface of the crystal where they would be more accessible to the stripping action of a suitable solvent. These considerations make it necessary to conduct separations of minor constituents from sucrose in aqueous solutions rather than from the dry sucrose. Liquid-liquid extraction is, at present, the most successful method for the quantitative measurement of minor constituents in cane sugar.

The efficiency of a liquid-liquid extraction of a sucrose solution is affected by a variety of parameters. These are the pH of the solution, Brix, the extraction solvent used, the ratio of extraction solvent volume to sucrose solution volume, and the length of extraction time.

The choice of the extraction solvent is very important. It has to be immiscible with water, but at the same time, capable of extracting one or more constituents quantitatively in a reasonable amount of time. Previous work on measuring the minor constituents in raw sugar (2)[2] had indicated that Brix had some effect on the quantity extracted. The other parameters are also expected to influence extractability. This report will summarize our findings to date regarding the effect of these parameters on extraction and the efficiency that is obtained with the methods employed.

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[2]Numbers in parentheses refer to items under "References" at the end of this paper.

Once the constituents are separated from the sucrose, they are identified and measured by gas-liquid chromatography. GLC is already in wide use throughout various segments of the sugar industry, especially for the measurement of sucrose (7) and invert (1, 9-12). Some work has been done on measurement by GLC of several of the well-known and commonly occurring constituents such as aconitic acid (8) and lactic acid (6). GLC is also being used successfully for the separation and identification of constituents in molasses (3, 13) and sirups (4).

GLC is an excellent method for measuring minor constituents as well as identifying them. The method is limited, however, to the study of compounds with a molecular weight range not exceeding 400-500 (5). Compounds to be studied by GLC are first converted to a volatile derivative and then partitioned through a column by a flow of gas; for this reason, very large, heavy molecules will not elute, except under extreme conditions of temperature and flow and then with only limited success. In general, polysaccharides, caramels, and melanoidins cannot be studied by GLC. Flavonoids, disaccharides, and trisaccharides represent the upper limit among the constituents in cane sugar that can be studied by GLC. The minor constituents of greatest interest to this study--those that are found in refined sugar, such as amino acids, organic acids, and fatty acids--are usually in the low molecular weight range and are well suited to analysis by GLC.

METHODS AND MATERIALS

Standard Extraction Procedure

A standard liquid-liquid extraction consisted of preparing solutions containing 50 g sugar at the required Brix, with adjustment to the desired pH with 0.2 N H_2SO_4 or 1 N NaOH. This solution was then extracted with several equal portions of ethyl acetate in a separatory funnel with vigorous shaking. In most cases, five portions of 50 ml each of ethyl acetate were used for extraction, except when the volume of the solvent was a parameter to be adjusted. These portions of extract were combined, dried with sodium sulfate, filtered, and prepared for gas chromatography by removal of the solvent under reduced pressure. The extracts were dried in a vacuum desiccator overnight.

Gas Chromatography

After the extracts were sufficiently dry, they were subjected to silylation with a silylating solution that contained a measured quantity of the internal standard, tetraphenylethylene (TPE), in Tri-Sil, which is a commercially prepared mixture containing the silylating reagent, a catalyst, and pyridine. An aliquot of this silylating solution was used to dissolve the entire extract. Once dissolved in Tri-Sil, it was fully derivatized and ready for analysis.

Gas chromatography was carried out on a Hewlett-Packard Model 5750. The column was a 6-foot stainless steel tube, 1/8 in. O.D., packed with 10% OV-1 on Chromosorb H.P., 80-100 mesh. Helium at a flow rate of 17 ml/min was the carrier gas. The injection port temperature was 270° C and the detector temperature was 300° C. A flame ionization detector was used.

A program was developed to give elution of all the constituents within 25 min with good separation and sharp peak shapes. Temperature programming consisted of a 4-min postinjection period at 100° C, temperature increase of 15° C/min to 270° C, and a 10-minute hold at 270° C.

Measurement of Constituents

Once the constituents in the extracts were identified, the relative response value, K, of each constituent relative to the internal standard, TPE, was determined. Solutions containing known weights of the constituent and the TPE were prepared and the resulting chromatograms analyzed. The calculation for K can be based on either the peak height or the peak area ratio of the two compounds, and the weight ratio, as given by the following equation:

$$K = \frac{\text{peak ht or area constituent}}{\text{peak ht or area TPE}} \times \frac{\text{wt TPE}}{\text{wt constituent}} \qquad (1).$$

The K values relative to peak area (K_A) as well as peak height only (K_{ht}) were determined for all constituents and a comparison of standard deviations and coefficients of variation showed in all cases that K_{ht} was subject to less variation than was K_A (see table 1). All calculations were, therefore, conducted on the basis of K_{ht}.

To calculate the weight of the constituent in an extract, a known weight of the standard was added and the following equation employed:

$$\text{wt constituent} = \frac{\text{peak ht constituent}}{\text{peak ht TPE}} \times \frac{\text{wt TPE}}{\text{K constituent}} \qquad (2).$$

The weight of the constituent, which was in mg/g of sugar, was converted to ppm for ease of comparison.

EFFECT OF pH

The first series of extractions was done on a light colored raw from Louisiana and both pH and Brix were varied. A total of seven solutions of this sugar were prepared, of 50 g each at 25 or 50 Brix, with pH acid, alkaline, or unadjusted for control. Table 2 describes the extracts and shows the weight of each extract before silylation. It is evident that the pH of solution determines the gross weight of material extracted.

Oleic acid, palmitic acid, and malic acid were identified and measured. Aconitic acid, glycolic acid, and lactic acid were also identified in the acid extracts but were not measured.

Table 3 lists the ppm acids extracted in each extract.

TABLE 1--Statistical analyses of K-values and a comparison of K_A vs K_{ht}

Compound		Mean	Standard deviation	Coefficient of variation (percent)	No. of trials
Mesaconic acid . . .	K_A	0.86	±0.041	4.7	3
	K_{ht}	1.46	±0.017	1.2	3
Fumaric acid	K_A	0.83	±0.129	15.5	5
	K_{ht}	1.51	±0.131	8.7	5
Malic acid	K_A	0.77	±0.079	10.3	12
	K_{ht}	1.46	±0.109	7.5	12
p-Hydroxybenzoic acid	K_A	0.88	±0.085	9.6	14
	K_{ht}	1.62	±0.098	6.1	14
Palmitic acid . . .	K_A	0.52	±0.121	23.3	11
	K_{ht}	0.80	±0.156	19.5	8
Oleic acid	K_A	0.38	±0.086	22.6	11
	K_{ht}	0.54	±0.106	19.6	11

TABLE 2--The Brix and pH of raw sugar solutions prior to ethyl acetate extraction, and the weight of the resulting extracts

Brix	pH	mg extracted
50	2.0	56.5
25	2.0	38.0
25	1.75	26.3
50	6.4	4.04
25	6.3	3.85
50	9.2	3.32
25	9.2	3.50

TABLE 3--The amount of constituents extracted from raw sugar solutions of varied Brix and pH

Extract Brix	pH	Palmitic (ppm)	Oleic (ppm)	Malic (ppm)
25	2.0	2.8	5.8	0.0
25	1.75	2.4	5.8	0.0
50	2.0	2.2	4.2	2.0
25	6.3	1.4	1.5	0.0
50	6.4	0.64	0.64	1.8
25	9.2	1.1	1.3	0.0
50	9.2	0.72	0.76	0.36

Examination of the data reveals several trends, which are clearly expressed in figure 1 for malic acid and figure 2 for oleic acid. Low Brix increases the extractability of the fatty acids and high Brix reduces extractability. Conversely, malic acid is more extractable at high Brix and extracted less at low Brix; no measurable amount was extracted from 25 Brix solutions at any pH. All constituents are more efficiently extracted from an acid solution.

EFFECT OF BRIX

A second series of extractions was done to elucidate further Brix effects. An Argentine raw sugar was used for these extractions. In this case, the pH was not adjusted and in all solutions was about 5.7. Four extractions were

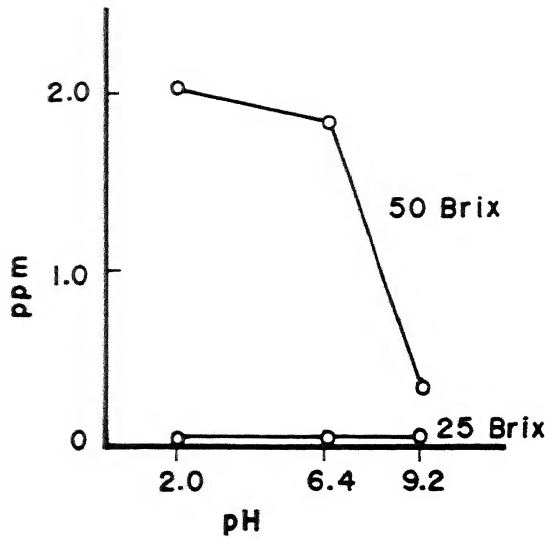

FIGURE 1--Effect of Brix and pH on extraction of malic acid from raw sugar.

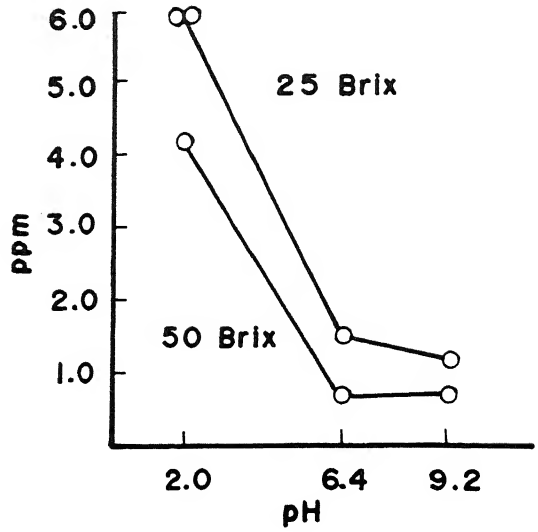

FIGURE 2--Effect of Brix and pH on extraction of oleic acid from raw sugar.

prepared from solutions of 10, 25, 50, and 60 Brix. The constituents measured for this sugar were malic acid, p-hydroxybenzoic acid, palmitic acid and oleic acid. The results are listed in table 4 and graphically represented in figure 3.

TABLE 4--Extraction of constituents from an Argentine raw sugar as a function of Brix of solution

Brix	Malic (ppm)	p-OHBA (ppm)	Palmitic (ppm)	Oleic (ppm)
10	2.56	3.5	11.3	27.5
25	8.25	6.7	12.7	30.7
50	19.8	8.8	15.8	24.4
60	34.1	9.2	4.2	8.1

The results bear out the findings of the first set of extractions. Maximum extraction of organic acids occurs at 60 Brix and of fatty acids at 25 Brix. Lowering the Brix to 10 does not increase extraction of fatty acids. It is to be expected that the amounts might have been somewhat higher had the solutions been extracted at acid pH.

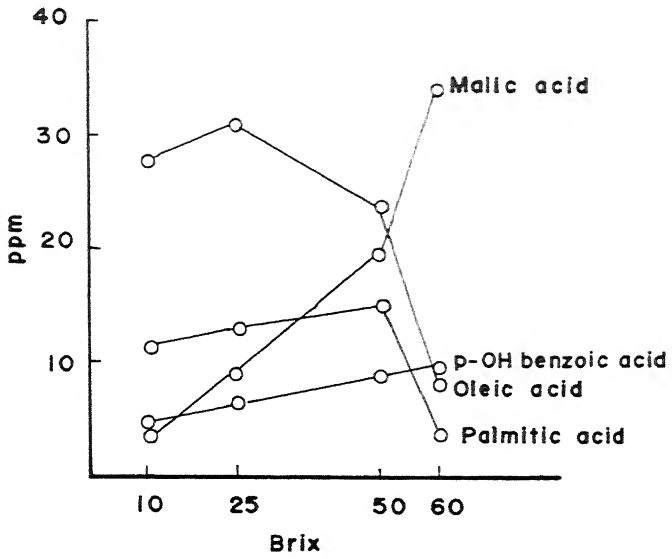

FIGURE 3--Extraction of constituents from Argentine raw sugar as a function of Brix.

EFFECT OF VOLUME OF ETHYL ACETATE

A third set of extractions was made to study the effect of the volume of solvent used during extraction on efficiency of extractions. A volume of 250 ml of ethyl acetate in five 50 ml portions was arbitrarily chosen for the development of a standard extraction method. Solutions of the Argentine raw were extracted with six 50 ml or 100 ml portions of ethyl acetate and each portion of extract was analyzed separately. The pH of the solutions was not adjusted and extractions were done from 25 and 50 Brix solutions. Table 5 gives the parameters of the extractions.

TABLE 5--Extraction parameters when amount of ethyl acetate was varied

Extract No.	Brix	Ml EtAc	Ratio (EtAc:Sucrose Soln)
1	50	50 ml x 6	300:80
2	25	50 ml x 6	300:180
3	50	100 ml x 6	600:80

Each portion of ethyl acetate extract was then measured separately for the four already identified constituents. The results are graphed in figures 4, 5, 6, and 7, where the summation of the amount extracted is shown as a function of Brix and volume of solvent. Several conclusions are suggested. For malic acid, it can be seen in figure 4 that doubling the volume of ethyl acetate increased efficiency of extraction; however, the slope of the extraction curve indicates that maximum extraction had not yet taken place, as the curve would be expected to plateau at that point with no further acid being extracted in subsequent attempts. For p-hydroxybenzoic acid, figure 5 shows that doubling the volume of solvent again improves extraction, but in this case maximum extractability is reached and the curve plateaus after the fifth extraction, with no further extraction of this constituent. In the case of the fatty acids, in figures 6 and 7, clearly, Brix is the determining factor in extraction. Doubling the amount of ethyl acetate at 50 Brix did not improve the extraction.

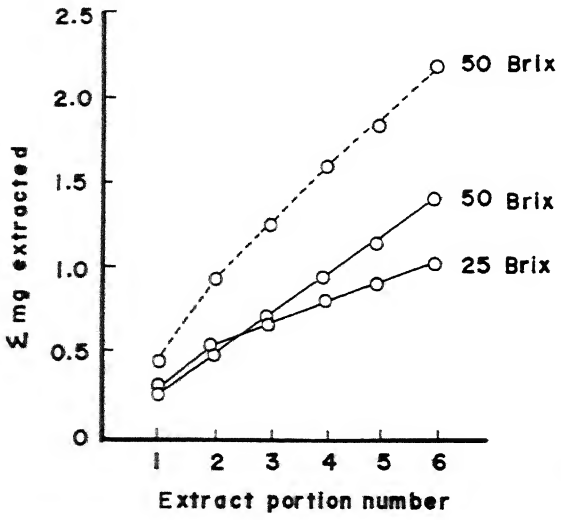

FIGURE 4--Extraction of malic acid from Argentine raw sugar showing summation of mg extracted in each portion of ethyl acetate. Broken line indicates doubling volume of ethyl acetate.

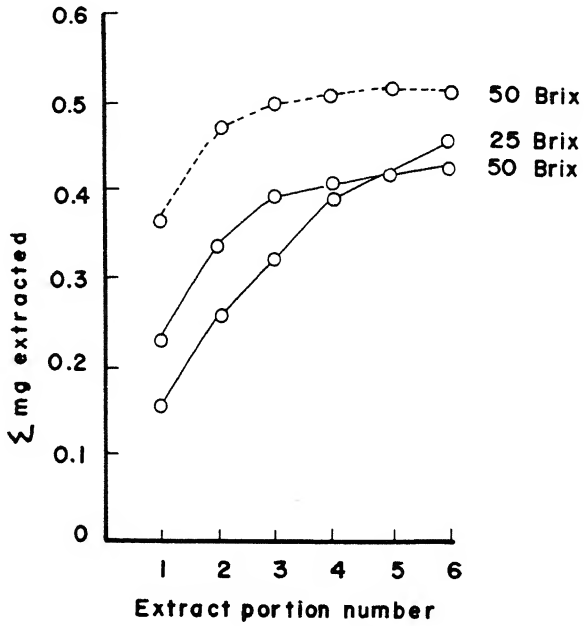

FIGURE 5--Extraction of p-hydroxylbenzoic acid from Argentine raw sugar showing summation of mg extracted in each portion of ethyl acetate. Broken line indicates doubling volume of ethyl acetate.

CONTINUOUS EXTRACTION

The results from the previous extractions (c.f., figure 4) indicated that not all the malic acid had been extracted with the methods used. Therefore, a continuous liquid-liquid extraction was done, affording the double advantages of essentially unlimited available volume of solvent and control over the time of extraction. A 50 Brix solution of 50 g of the Argentine raw, pH unadjusted,

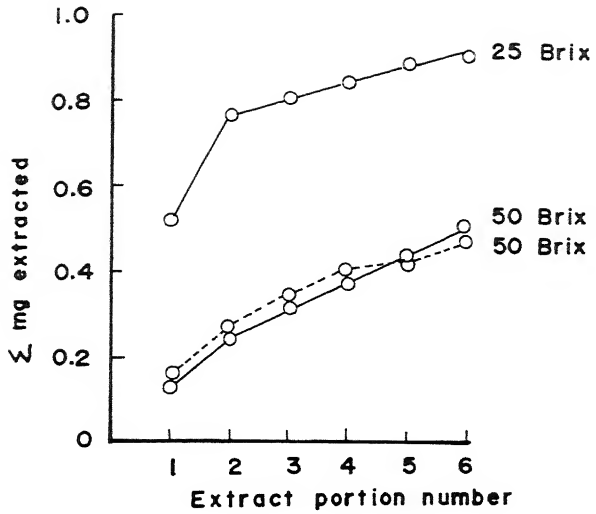

FIGURE 6--Extraction of palmitic acid from Argentine raw sugar showing summation of mg extracted in each portion of ethyl acetate. Broken line indicates doubling volume of ethyl acetate.

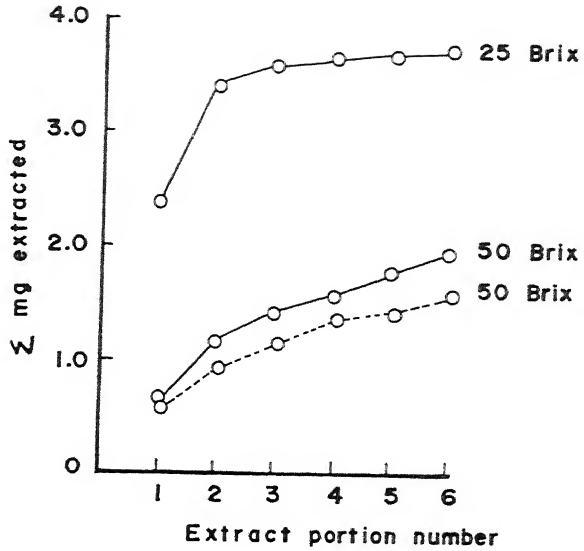

FIGURE 7--Extraction of oleic acid from Argentine raw sugar showing summation of mg extracted in each portion of ethyl acetate. Broken line indicates doubling volume of ethyl acetate.

was continuously extracted with 250 ml ethyl acetate for 20 h. The resulting extract was analyzed; table 6 gives these results along with a comparison of the maximum amount of each constituent obtained in previous extractions in separatory funnels, described earlier in this report.

TABLE 6--Comparison of amounts extracted from Argentine raw during a continuous liquid-liquid extraction with maximum amounts extracted in separatory funnel extractions

Constituent	Cont. ext'n (mg)	Sep. funnel ext'ns (mg)
Malic acid	4.42	2.11
p-OH benzoic acid	0.480	0.511
Palmitic acid	0.735	0.900
Oleic acid	2.39	3.69

Although less of any other constituent was extracted, at least twice as much malic acid was extracted by the continuous extraction method. This extraction was carried out at 50 Brix and did not, therefore, represent optimal conditions for the extractions of the fatty acids.

The solution of sugar extract was then adjusted to pH 2.0 with 0.2 N H_2SO_4 and continuous extraction with ethyl acetate was repeated. GLC measurement showed that an additional 18.3 mg of malic acid had been extracted. The other constituents were no longer visible on the chromatogram; they had, therefore, previously been fully extracted.

This type of step-wise extraction procedure would be indicated in cases where one or more constituent was present in a much larger amount than others, as in this case is malic acid. Aconitic acid would also probably have to be measured this way on GLC. It would first be necessary to try to extract the less concentrated constituents and then to adjust the conditions for optimum extraction of the more concentrated constitutent.

A problem that develops with a low pH continuous liquid-liquid extraction using ethyl acetate is that excessive invert is extracted. These sugars require a large volume of reagent to silylate and can consequently ruin the chromatography of the other constituents by overdilution. This is a difficult problem to overcome because ethyl acetate is an excellent solvent for this type of work, and other than for low pH continuous extractions is usually adequate.

Ethyl ether was substituted for ethyl acetate, but was found to be less efficient. It did have the advantage that little invert was extracted. However, a survey of ether extracts of various sugars revealed a fourth strike refined that contained measurable quantities of fumaric and mesaconic acids. Ethyl ether was the preferred extraction solvent for both of these acids. Mesaconic acid was not extracted by ethyl acetate, and fumaric acid only slightly.

Table 7 gives a list of the constituents that were tested for in both ether and ethyl acetate extracts of the same sugar. Chlorogenic acid and sinapic acid are listed as inconclusively identified because sinapic acid elutes within the range of invert, and chlorogenic acid elutes very late and needs to be concentrated more. However, high voltage electrophoresis was negative for both constituents in both extracts.

To measure the fumaric and mesaconic acids in this sugar, a continuous liquid-liquid extraction of 80 g of sugar in a 50 Brix solution at pH 2.2 was carried out with 1 lb ether. The extract was prepared for GLC in the usual manner. K-values for fumaric acid and mesaconic acid were determined to be 1.51 and 1.46, respectively (c.f. table 1). The extract yielded 2.53 ppm fumaric acid and 0.43 ppm mesaconic acid.

In all the methods so far described in this report, conditions have been determined that will remove as much of a particular constituent as can be extracted under optimum conditions. This does not, however, necessarily indicate that 100% of the constituent is extracted, even under optimum conditions.

The degree of extraction of fumaric acid was determined by carrying out duplicate recovery trials. The same continuous extraction procedure was set up, and 1.11 mg of fumaric acid added to the solution prior to extraction. Analysis showed 50% recovery in both cases. The consistency of recovery suggests that the determination and use of a recovery function in analysis can be a useful method for accurate measurement of the minor constituents in sugars.

TABLE 7--Constituents in a fourth strike refined sugar

Tested for	Results[1]
Succinic acid	++ (Too small to measure)
Mesaconic acid	+ (Ether only)
Fumaric acid	++
Malic acid	++
p-OH benzoic acid	+ (EtAc only)
Aconitic acid	-
Citric acid	-
Palmitic acid	+ (Ether only)
Syringic acid	-
Quinic acid	-
Caffeic acid	-
Chlorogenic acid	? (Inconclusive)
Sinapic acid	? (Inconclusive)
Glycolic acid	++
Lactic acid	++
HMF	++
Oxalic acid	++ (Too small to measure)
Levulinic acid	++ (Too small to measure)

[1] ++ means positive results with both solvent extractions.

CONCLUSION

In theory, other methods of determining constituents in sugars, such as fermentation and ion exchange, are preferable to solvent extraction because these procedures theoretically obtain all the constituents free from the sugar without solvent partitioning effects. In practice, in this laboratory, these methods have not yielded satisfactory results, but the liquid-liquid extraction methods have been consistently reproducible. It is now possible to decide on a set of conditions that are optimal for individual constituents on the basis of their chemical structure, by utilizing liquid-liquid extraction of a sugar solution at suitable pH and Brix with a solvent chosen to give a good extraction. The procedure may include a step-wise extraction as was necessary for malic acid and must be supplemented by the determination of a recovery function for each constituent.

The appendix lists the conditions used for extracting and measuring the minor constituents herein reported.

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Appendix--Optimum extraction conditions for some minor constituents in commercial cane sugars

Malic acid

50-60 Brix solution; pH 2.0; continuous liquid-liquid extraction for at least 10 h with ethyl acetate.

p-Hydroxybenzoic acid

50 Brix solution; pH 2.0; separatory funnel extraction with six equal portions of ethyl acetate in ratio of 12:1 ethyl acetate:water; pH does not need to be adjusted to the acid side, because same results are obtained when pH is not adjusted, along with less trouble with invert and other acids overpowering the p-hydroxybenzoic acid.

Palmitic acid

25 Brix solution; pH 2.0; separatory funnel extraction using six equal portions of ethyl acetate in a ratio of 2:1 ethyl acetate-water.

Oleic acid

Same conditions as for palmitic acid.

Fumaric acid

50 Brix solution; pH 2.0; continuous liquid-liquid extraction with ethyl ether.

Masaconic acid

Same as for fumaric acid.

DISCUSSION

J. F. DOWLING (CPC International): Have you tried any method other than retention times to verify your peaks? Have you tried thin-layer chromatography, or infrared?

M. A. GODSHALL: We have tried thin-layer chromatography, high voltage electrophoresis and some fluorescence studies.

J. F. DOWLING: What sort of detector do you use? What about the response factors?

M. A. GODSHALL: We use the flame ionization detector. Response factors are determined using an internal standard.

J. F. DOWLING: Do you get a one-to-one response?

M. A. GODSHALL: No, that's why we use the internal standard. Before we start to measure these constituents, we work with known amounts of the compounds mixed with known amounts of internal standard--in our case, tetraphenylethylene--and we determine what the response factor of each compound is.

J. F. DOWLING: Then we do have this data available to us?

M. A. GODSHALL: Yes.

J. F. DOWLING: Good; that involves a good deal of work to determine the response factors--and you have done that for us all.

V. S. VELASCO (CPC International): What is the retention time for invert sugar? Does it interfere with the fatty acid peaks?

M. A. GODSHALL: No; with the program and the column that we use, the palmitic acid elutes a little further out than the last glucose peak. (As you know, glucose has α and β peaks.) There is no overlap: the peaks are touching, but you can see that one peak is glucose, and the other peak is palmitic acid. Oleic acid elutes quite a bit past the glucose, so there is no confusion.

M. MATIC (Sugar Milling Research Institute): If you were to use capillary columns, you could achieve better separation, and possibly omit some of the prior separations. Of course, there may be so many peaks that it may be impossible to identify them. But if it were possible, you could cut quite a lot of working time. Also, with your partition methods, you can never be quite sure that you have extracted the total amount of any one compound.

M. A. GODSHALL: Are you suggesting that we put in the whole sugar?

M. MATIC: We are doing similar work with kestose, a trisaccharide which comes out much later. With columns of 300 or 400 ft, you could get quite a lot of compounds in 60 to 90 minutes. Also, you could do the work in water, which would simplify matters.

M. A. GODSHALL: We don't have that type of equipment yet, but using water certainly would make things easier.

W. L. REED (Revere Sugar): Can this method of isolation be used on any of the minor constituents?

M. A. GODSHALL: This is a very interesting problem: if someone were concerned with a particular compound, then we would try to develop conditions for that compound using what we already know to determine conditions optimum for extracting the particular compound.

F. G. CARPENTER: If a whole sugar is derivatized and run through a column, there are at least 80 peaks. With 80 peaks, it is difficult to identify them with any certainty. A more efficient column might get more than 80 peaks.

That is the reason that we are trying to cut down on the number. Also, we are at present interested in the compounds that elute before sucrose.

We could, and eventually will, develop conditions for determination of many other minor constituents that may be important, but for the present we are sticking to only a few at a time.

K. R. HANSON (Amstar): Joe, you said that a poor sugar flocs heavily; Mary An says she can identify these minor constituents that are in poor sugar. Can't we tie these two things together?

J. F. DOWLING: Mrs. Godshall didn't say she could identify every constituent. She can identify quite a few qualitatively, and these ones she's talked about today quantitatively, on GLC, but none may be tied to floc.

F. G. CARPENTER: The scheme is that, as fast as Earl thinks up a new compound that might be important to look for, Mary An figures out a way to measure it.

J. F. DOWLING: How would you measure the polysaccharide that may be involved in floc formation?

M. A. GODSHALL: By gas-liquid chromatography; we measure the hydrolyzed polysaccharide in a similar fashion to this work.

C. C. CHOU (Amstar): Have you used high pressure liquid chromatography?

M. A. GODSHALL: That is a very good technique, particularly for higher molecular weight compounds, but we are not using it for these smaller molecules.

CANE SUGAR AND SILICON COMPOUNDS

By Margaret A. Clarke[1]

ABSTRACT

The roles of silicon compounds in cane sugar production are reviewed. Their effects on cane growth, inversion control and filtration are considered. The possible role of silicon in beverage floc formation is discussed.

INTRODUCTION

Silicon is an element that plays many roles in sugar production, but its presence and interactions with sucrose and cane sugar constituents are often overlooked.

The refiner may think of silica (silicon dioxide) when it clogs his filter cloths, or when it is a major component of inorganic ash, but otherwise silica has been of little importance to refiners.

Silicon compounds have been shown to be important in regulation of cane growth (1, 10, 11, 14, 15)[2], in control of inversion in cane juice in factory clarification and filtration (2-4), and in refinery filtration (5). Because there are so many areas of sugar production where silicon compounds now are involved, and because they may be involved in the formation of beverage floc, it is worthwhile to review the uses and functions of this element in cane sugar production and to show how it could be important in floc formation.

Some sources of silicon, by which it may enter process liquors, are: soil, cane plant, growth regulators, antifoam additives, anti-inversion additives, and flocculation aids.

In field work, it has been known for some years that application of calcium silicate to soil will increase production of cane, and percent sucrose in cane, the latter possibly by increases in sucrose level in cane leaves.

In pot culture, when a series of elements is added to the soil, silicon in plant and soil is better associated with dry weight increase than is any other element tested (10, 11, 14, 15). Silicon treatment with various silicate salts has variously been reported, in production of several tropical crops, to increase oxidizing power of roots, produce more erect leaves, and enhance resistance to diseases and pests (10). The mode of action, however, in these instances and in its role in increasing sugar production remains obscure. The

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[2]Numbers in parentheses refer to items under "References" at the end of this paper.

inhibitory effect of silicon on invertase formation is probably somehow connected with the effect of silicon on plant growth. It has been observed that percent silicon in cane increases with the age of the plant (20), and it is becoming increasingly evident that silicon is an essential element for plant growth.

INHIBITION OF INVERTASE

Some years ago, it was found that the addition of silicon, in the form of sodium metasilicate (so-called), to cane juice, retarded sucrose degradation (1, 2). The action was thought to be through inhibition of invertase activity. Indirectly, organic acid synthesis was also suppressed because formation of the substrates (the hexoses in invert) required for bacterial growth was lowered. Levels of silicate of 40 to 60 µmoles/ml gave complete inversion control for 48 to 60 h, and partial control up to 96 h. Alexander and coworkers in Puerto Rico, who have done most of this invertase work, report (3, 4) that upon addition of sodium metasilicate in amounts > 20 µm/ml to raw juice, juice darkens, pH rises, flocculation of suspended matter occurs, and sucrose is retained intact for 36 h. Paper chromatographic procedures as well as polarization were used to trace the state of the mixture. Storage of juice for longer periods without inversion required the addition of a greater amount of silicate, and when 50 µm/ml were added, neither sucrose nor pH values decreased over 48 h.

Because of the pH increase from addition of the highly basic sodium salt, and its possible effect on inversion rate loss, juice samples were adjusted to pH 7 and pH 5.6. These samples showed inversion delay in the same degree as those without adjusted pH. The conclusion was that silicate action involves directly either invertase or its substrate, sucrose. Alexander *et al.* (4) proposed that the mode of action was through a silicate-sucrose complex, rather than by the essentially physical process of entrapment, by a silicic acid gel, of enzyme-substrate complex. Microbiological experiments showed the possibility of linkage of silicate with the fructose end of the sucrose molecule. An additional observation was that the floc formed upon addition of silicate removed a good deal of silicate from solution. A prior filtration of the cane juice lowered the silicate requirement by a factor of ten, thus indicating the involvement of silicate in clarification floc formation.

In clarification of cane juice, activated silica can be used as a clarifying agent (22), but its price is prohibitive. It has the advantage of clarifying well at lower temperatures than lime. Silicate that is already in cane juice is quite well removed by lime clarification and filtration; if not removed, it ends up in the crystal (12).

FILTERABILITY

There has been some discussion about the influence of silica content on filtration. The majority of workers, including Alexander in South Africa (5), who found that soluble silicate and wax are important factors in filtration, and people in Taiwan (9), Japan (24), and India (18), agree that increased silicate content decreases the efficiency of rate of filtration. The Japanese

workers, however, in a study on filtration of carbonatation slurries, found almost no correlation of filterability with silica content.

In a discussion of filtration in refineries (8), Bennett found that, in carbonatated liquors, silica content increased with increasing lime dosage, over about 0.6% of lime. Since calcium content also increased, although less rapidly, the change is presumably due to a difference in the type of precipitate formed that occludes the silica, and not from some silicate impurity in the lime.

Some other uses of silicon compounds, which are, or might be, of interest to the sugar industry, are the use of magnesium or calcium aluminum silicate salts to prevent caking in free flowing powdered foods, such as powdered sugar (16), and the use of activated silica in waste water coagulation, particularly in waste waters of low alkalinity (23).

NUTRITION

As far as silicon in foods is concerned, silicon is not toxic, but has not yet been shown to be essential for man, although present evidence indicates that it may well be essential. Silicon is essential for rats and chicks, for early bone structure formation and general growth (17).

ANALYTICAL METHODS

The basic analytical method for silicon has, for many years, been the spectrophotometric reading of the yellow silica molybdate complex at 400 nm (20) or the blue complex at 650 nm (21). This method which determines soluble silicates, and not insoluble silica, is usually sufficiently accurate for sugarcane, cane juice and raw sugars and adequate even for refined sugars. However, some of the newer instrumental techniques, e.g., atomic absorption spectrophotometry, give greater accuracy for low levels of soluble silicates below 10 ppm, such as those in refined sugars. Table 1 shows some values for silicon in cane stalk (10), cane juice (19) and raw sugar (6, 19). Atomic absorption spectrophotometry can be used to determine total silicon.

TABLE 1--Silicon levels

Source	Si (ppm)
Cane stalk	≃1000
Cane juice	30 - 200
Raw sugar	10 - 100

SILICATE STRUCTURE

Silicon is a very common element composing about 28% of earth's crust, second only to oxygen, with which it is usually found in combination, as silica or silicates. The bonding of the silicon, in silicates and most other compounds is sp^3 tetrahedral, as shown in figure 1, like that of carbon. The single bonds of silicon have considerable ionic character, more so than those of carbon, and silicon has very little tendency to form multiple $p\pi$ bonds.

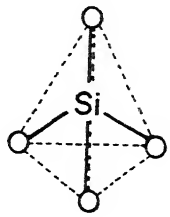

FIGURE 1--Structure of SiO_4^{4-} tetrahedron, as in sodium silicate, $Na_mH_{4-m}SiO_4$ where $0.64 < m < 1.04$.

Figure 1 shows the general formula for sodium silicate, which is usually a mixture of hydroxysilicates with SiO_4^{4-} tetrahedra as the basic units (7). Although most silicates are polymers, there are a few silicate salts with discrete SiO_4^{4-} ions; in these, e.g. Zn_2SiO_4, the coordination is through the oxygen (13).

In all figures, the SiO_4 tetrahedral configuration is represented two-dimensionally, for diagrammatic purposes. Each silicon is bonded to four oxygens, with variations in the sharing of oxygen atoms in different types of polymers.

Cyclic Anions

The smallest polymeric silicates are the cyclic anions, of which only two forms are known. In figure 2, $Si_3O_9^{6-}$ is one form, found in $BaTiSi_3O_9$, benitoite. The other larger anion, $Si_6O_{18}^{12-}$ is found in beryl, $Be_3Al_2Si_6O_{18}$.

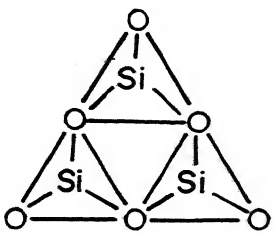

FIGURE 2--Cyclic anion $(Si_3O_9)^{6-}$.

Infinite Chain Anions

Next in complexity, and probably more of importance to us, are the infinite chain anions. Figure 3 shows one of the two chain types, the pyroxene type. Chains of $(SiO_3^{2-})_n$ lie parallel (13) and are held together by cations between the chains. Many minerals, e.g., enstatite and diopside, are in this category. The silicates in beverage floc could be of this type, with long chains held together by cations. The specific identity of the cations is not important for this structure (there is lots of space for most cations) as long as the total positive charge is sufficient to produce electroneutrality. This is the structure of the so-called "metasilicate." There is no "metasilicate" anion or "metasilicic acid," per se.

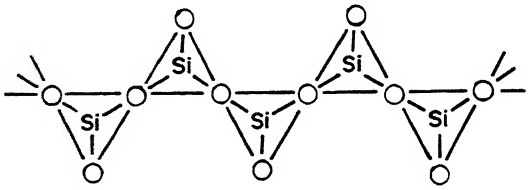

FIGURE 3--Infinite chain anion, pyroxene type, $(SiO_3^{2-})_n$.
Na_2SiO_3 is "metasilicate".

Another type of chain structure, shown in figure 4, is the amphibole structure: long, parallel bands, again held together by cations. Asbestoses and tremolite, $Ca_2Mg_5(Si_4O_{11})_2(OH)_2$, have this type of structure. Apparently there are always some hydroxyl groups attached to the cations in the amphibole structures.

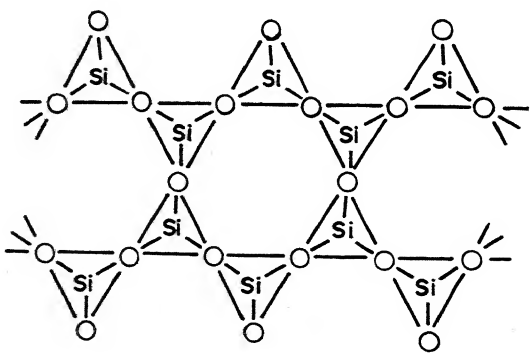

FIGURE 4--Infinite band anion, an amphibole type, $(Si_4O_{11}^{6-})_n$.

Infinite Sheet Anions

Figure 5 shows a type of infinite sheet silicate anion, with an infinite two-dimensional network of formula $(Si_2O_5^{2-})_n$. The sheets are held together by cations; micas have this structure.

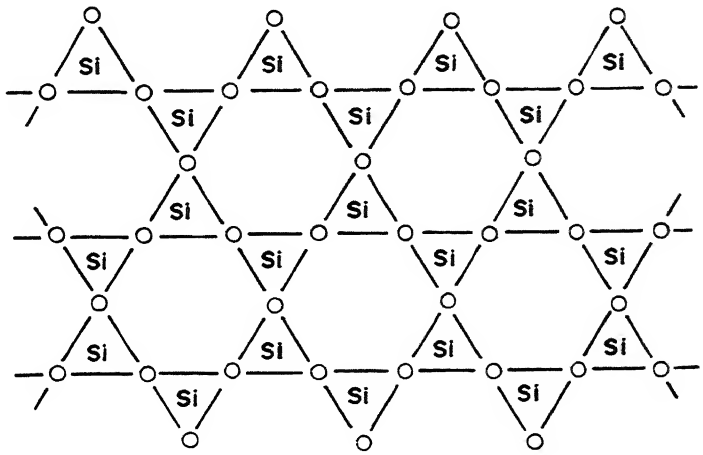

FIGURE 5--Infinite sheet anion $(Si_2O_5^{2-})_n$.

Framework Minerals

There are other, more complicated, silicates with three-dimensional structures in which every oxygen is shared by two tetrahedra. That empirical formula would be $(SiO_2)_n$ with SiO_2 units of zero valency. However, in nature, some silicons are replaced by aluminum ions, to form a charged unit. This is the type of framework structure that makes up molecular sieves and some ion-exchange resins.

BEVERAGE FLOC

It seems possible that some of these long chain silicate ions exist in sucrose solutions, and that they may be one factor responsible for floc formation. Work at our laboratory has shown that it is possible to get floc formation in formerly nonflocccing sugars by addition of silicate salts under various conditions. There is known to be conversion between forms of silicate as the pH is lowered, and a complex between silicate and sucrose is quite likely: figure 6 shows a sucrose molecule and the structural formula for "metasilicate," where it is evident that the possibilities for a borate-sucrose type of complex are excellent. It is not claimed that silica or silicates give all the answers to the floc problem, but it appears that they probably are a factor in floc formation. A third factor may be required to permit floc to form.

In conclusion, it appears that silicon and its compounds play enough roles in the manufacture of cane sugar to merit a review of this element's place in the sugar industry.

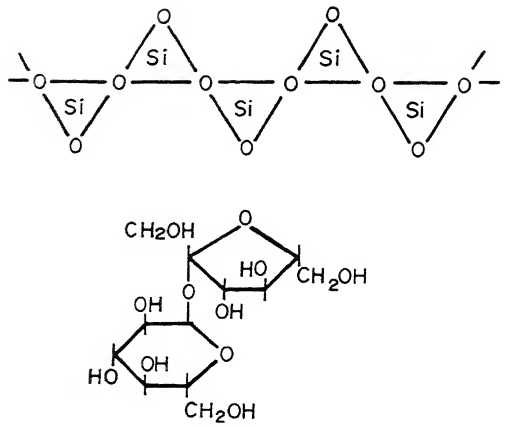

FIGURE 6--Possible interaction between silicate chain anion and sucrose molecule.

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DISCUSSION

W. R. TUSON (Colonial Sugars): Earl Roberts showed that there was about 70% silica in his floc. You indicate that silicon compounds may be involved in floc formation. Earl also talked about the involvement of organic compounds in floc formation. Is there more to this story?

E. J. ROBERTS: Yes, I have done a little more work in this line. I found that some of the sample sugars we had that would not by themselves form an acid floc would floc if we added sodium silicate to them before acidifying; if, that is, the sugar also contains an alcohol-precipitable polysaccharide. We have a sample of sugar which contains no alcohol-precipitable polysaccharide, and that would not floc at all even with added silicate. We have to look further at this combination of polysaccharide and silicate in nonfloccing sugars.

J. F. DOWLING (CPC International): If you keep a beverage around long enough--for two years say--it will usually floc. Could this be due to silica dissolving in from the glass container?

M. A. CLARKE: Yes, silicates could possibly go very slowly into solution at the low pH's found in most beverages.

C. C. CHOU (Amstar): Did your method of analysis differentiate between silicates, which are soluble, and silica, which is not?

M. A. CLARKE: The figures in the paper were not found by us; they are from the literature and are all soluble silicates, I believe, determined by the spectrophotometric method. An additional digestion of the sample with hydrofluoric acid is required to dissolve silica.

M. MATIC (Sugar Milling Research Institute): From your evidence for floc formation, do you think that the silicate polymer is formed slowly, or does the polymeric species react slowly with the polysaccharide that is also present? What conformation of the polymer do you think is present?

M. A. CLARKE: I hope that by the next Technical Session we will have some answers for you. I would hesitate to guess which polymeric silicon-oxygen conformation would be present. We're trying right now to figure out the factors that determine involvement of a silicon-oxygen compound--then we'll look at what compound it might be and where it might come from--what possible sources there are--and if a third factor is necessary.

J. F. DOWLING: One thing you need to have is a low pH. You're measuring the floc visually, over a period of days, so you're really looking at a concentration gradient. However, we still don't know the key that sets it off. The silicate plus polysaccharide idea sounds quite promising; the acid pH is necessary too.

M. A. CLARKE: Perhaps the low pH is required in order to have the silicate in the right form, or perhaps to begin hydrolysis of the polysaccharide and make it better available for complex formation.

J. F. DOWLING: From some of the data we've seen today, there appears to be high silica floc--the kind that Earl isolated--and low silica floc--the kind we have found. However, there is silica in all of them. I wonder if that means that it is an essential for floc formation or that it coprecipitates readily.

R. S. PATTERSON (California and Hawaiian): It appears that silica is beneficial in the early stages of sugar production as a growth stimulant, but harmful in the later stages, in filter clogging and floc formation. It would be good to hit a happy medium.

CHLORIDE CONTROL IN THE REFINERY

By C. R. Brown[1] and P. Pommez[2]

(Presented by C. R. Brown)

ABSTRACT

Reasons are presented for the selection of chloride as a primary control in the refinery. Available systems of chloride control, and typical uses in the plant, are discussed. Experiences with the use of chloride control in affination and in pan back boiling are presented.

INTRODUCTION

The availability of chloride and sodium ion-selective electrodes now makes possible the monitoring of these ions in process streams. It was realized early that whichever ion-selective electrode, or selectrode, was selected for use in the refinery, it must be accurate, reproducible, and easily checked by a laboratory technician using a relatively simple analytical method. The chloride ion electrode satisfies these requirements; consequently, it was decided to develop the system and logic required for chloride control by the ion-selective electrode.

SYSTEMS OF MEASUREMENT

The theory behind the chloride electrode and its response in a sucrose solution has been thoroughly documented in a paper presented to this organization[3]. This paper also reported the preliminary results of the laboratory investigation of a measurement system using an ion-selective electrode in conjunction with a double-junction reference electrode, conducted at Redpath's Montreal refinery. At that time, it was decided to investigate a system where a chloride ion-selective electrode was substituted for the reference electrode, since the reference electrode is known to be a potential source of instability in the system.

Two other alternative systems were briefly considered. One made use of a wood reference electrode where streaming potentials are supposedly eliminated,

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[2]Chief Chemist, Redpath Sugars, Ltd., P. O. Box 490, Montreal, Quebec, Canada H3C 2T5.

[3]Pommez, P., and Stachenko, S. 1970. Use of chloride electrodes in refinery control. Proc. Tech. Sess. Cane Sugar Refin. Res. 1970: 82-102

developed by Betz Laboratories[4], and the other is the tag-ion system developed at Orion[5] where two ion-selective electrodes are used, which are not necessarily of the same species.

No comment will be made here on these latter two systems, since we have not conducted any study on them and have limited ourselves to the two systems first mentioned.

APPLICATIONS

From a theoretical point of view, there are a number of obvious places in the refinery where the chloride ion-selective electrode could be used to control the process operation:

1. Affination

2. Sirup back boiling

3. Char or carbon sweetening-off

4. Scum and mud sweetening-off

As mentioned above, there are many possible systems for measuring chlorides, and there are also several approaches to monitoring the ion, depending on a number of factors. In the case of Redpath Sugars, the fact that one refinery has an on-stream computer made possible some refinements in data exploitation. On the other hand, the absence of such a commodity in the other refinery forced a totally different, though similarly successful, approach. The two operations which have been explored for control to date are affination control, and jet 1 (first strike sirup) back boiling.

AFFINATION

As has been reported elsewhere[6], studies connected with the installation of an on-stream computer in Redpath's Toronto refinery showed that the theoretical exclusion of chlorides from the crystal could be practically applied as a means to control the efficiency of the affination operation. Percent removal of chlorides by affination is compared in table 1 with removal of other non-sucrose constituents.

The system developed in the Toronto Refinery is as follows:

1. A single affination machine can be controlled by the on-stream computer to operate with varying washes through five successive cycles.

2. Representative samples are manually accumulated from each of these five cycles and analyzed for chlorides and invert.

[4]Betz Laboratories, Inc., Trevose, Pa. 19047.

[5]Orion Research Laboratories, Inc. 1970. The ABC's of monitoring. Orion Research Newsletter 3: 21-23.

[6]Baker, K. E., and Stachenko, S. 1970. Use of a process control computer in a cane sugar refinery. Proc. Sugar Ind. Technol. 29: 166-175.

3. The results of these analyses are introduced to the computer which submits them to a regression analysis.

4. The computer prints out the optimum wash for the raws being processed at that time and also predicts the corresponding invert level.

TABLE 1--Laboratory affination of raw sugar. Percent removal of non-sucrose constituents

Constituent	Mean % removal	Standard deviation
Chlorides	89.59	3.89
Ash	76.17	7.93
Invert	73.80	11.62
Color	75.75	7.53
Calcium carbonate	71.65	8.16
Potassium	84.41	6.02
Sodium	80.40	5.42
Starch	47.47	20.13
Gums	65.21	27.97

In the automation of such a system, the logic was to have an automatic composite sample of washed raw sugar from the single computer-programmed centrifugal machine, melt that sample and automatically measure the chloride, and feed this data to the computer to coordinate the chloride reading to a particular wash. The schematic for this sampling procedure is shown in figure 1, and the sample assembly is diagrammed in figure 2. The system failed to work on a long term basis because of the difficulty of getting a consistently representative sample of washed raw sugar. Continuing work is being done to develop a reliable sampling system. It should be noted that the system shown does permit sampling, dissolving, system purging and a stable reading before the next load from the same machine is dumped and has to be sampled.

An alternative system was used in the Montreal refinery, because their situation was quite different, in that there is no on-stream computer, and remelt sugar is dissolved in a separate melter. It was decided to sample the washed raw sugar liquor and monitor the chloride in it.

After successful laboratory experimentation (which will be reported elsewhere) the technique of differential measurement was adopted to monitor the chloride content of the washed raw sugar liquor. The differential measurement uses two chloride electrodes: one gives a fixed reference potential by measuring a fixed chloride content in a reference liquor, the other measures chloride in the washed raw sugar liquor.

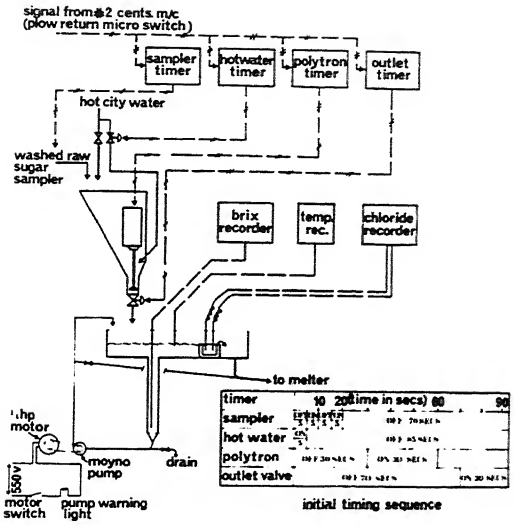

FIGURE 1--Sampling scheme for washed raw sugar at Toronto refinery.

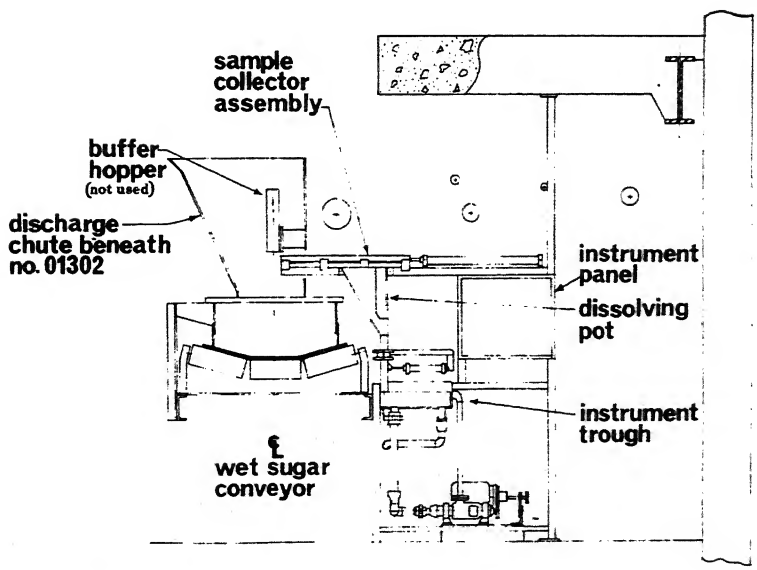

FIGURE 2--Sampling assembly for washed raw sugar at Toronto refinery.

Instrumentation

A schematic view of the system used at the Montreal refinery is shown in figure 3. Several problems arose as the system was put to test, and were worked out accordingly.

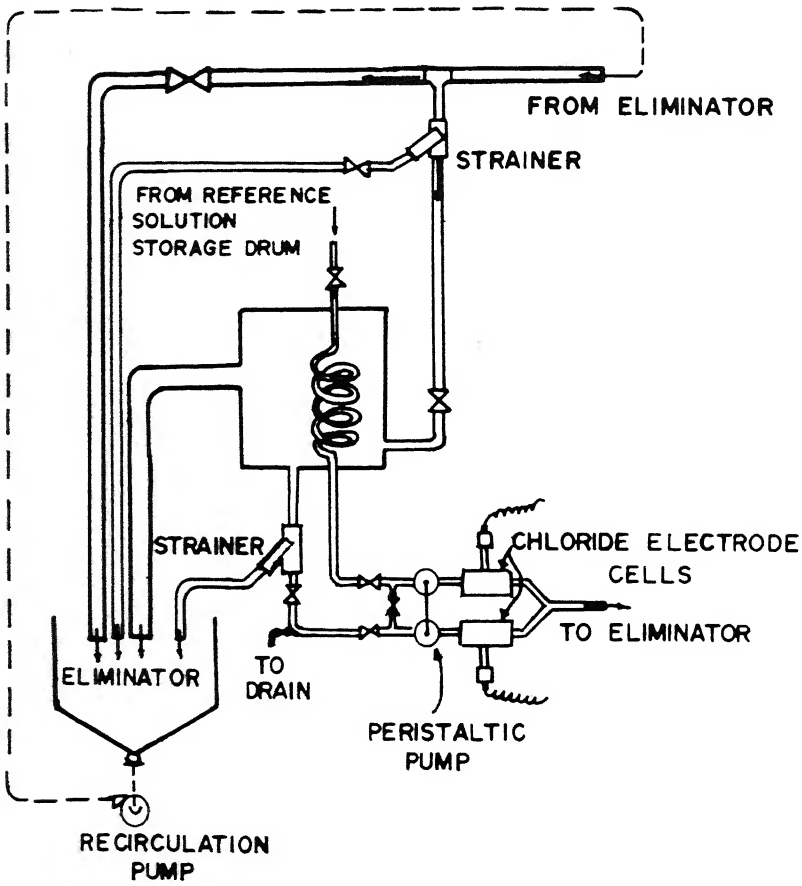

FIGURE 3--Chloride measurement system for washed raw sugar liquor at the Montreal refinery.

Ionic Strength of the Solutions

It is important that both reference liquor and sample liquor have the same ionic strength (the electrode measures activity and not concentration), and also that the reference liquor contains a fixed chloride content. The ionic strength of a washed raw sugar liquor is close to 10^{-1} M, while fine liquor used for the reference solution is about 10^{-3} M. Adjustment of both ionic strength and chloride level in fine liquor was achieved by adding a fixed amount of sodium chloride to fine liquor when preparing the reference solution. The activity coefficient of both solutions is then the same and close to 0.80, as shown in figure 4.

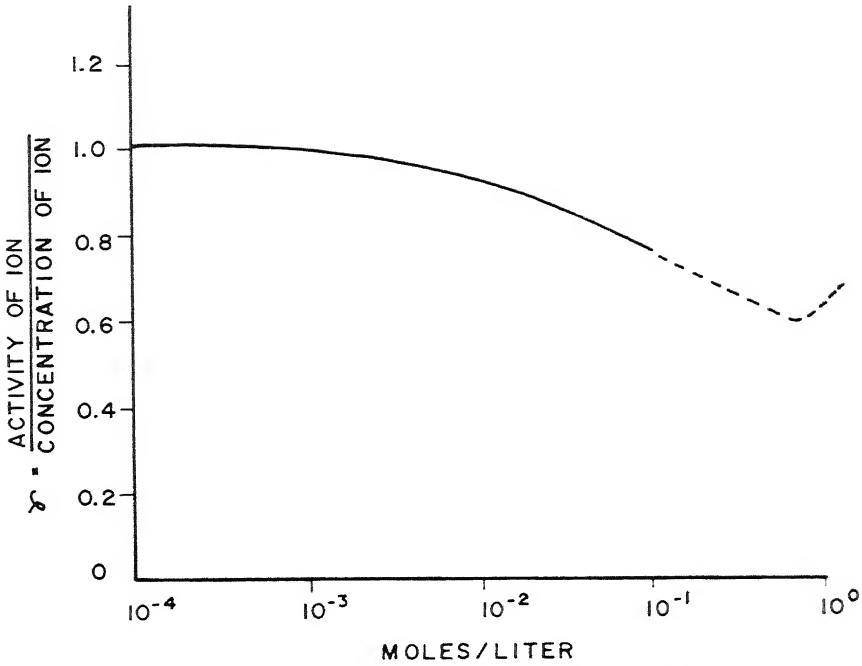

FIGURE 4--Variation of chloride activity with total ionic strength of solution.

Brix Sensitivity

The Brix of fine liquor is about 67.5 and that of the washed raw sugar liquor fluctuates between 68 and 68.5. Even with such a fluctuation the reproducibility of the measurements was still acceptable.

Temperature Compensation

The effect of temperature on the electrode response is important in two respects:

1. The theoretical slope of the curve mV vs concentration for any electrode varies drastically with the temperature.
2. Both reference and sample electrodes must be at the same temperature.

Temperature equilibrium was achieved by having the reference liquor flow through a coil immersed in the sample tank. Thus any change in melter temperature--generally due to changes in atmospheric temperature--will affect both liquors to the same extent. The sampling arrangement is shown in figure 5.

Flow Conditions

The flow rate should be identical and constant in the two electrode cells. This state was achieved by using a peristaltic pump. However, the pulsing characteristic of this pump introduces fluctuations in the mV meter. A timer was put in to stop the pump every 4 to 5 minutes for 30 seconds. A stable digital reading is reached in about 15 seconds. The pump and the chloride measurement cells are shown in figure 6.

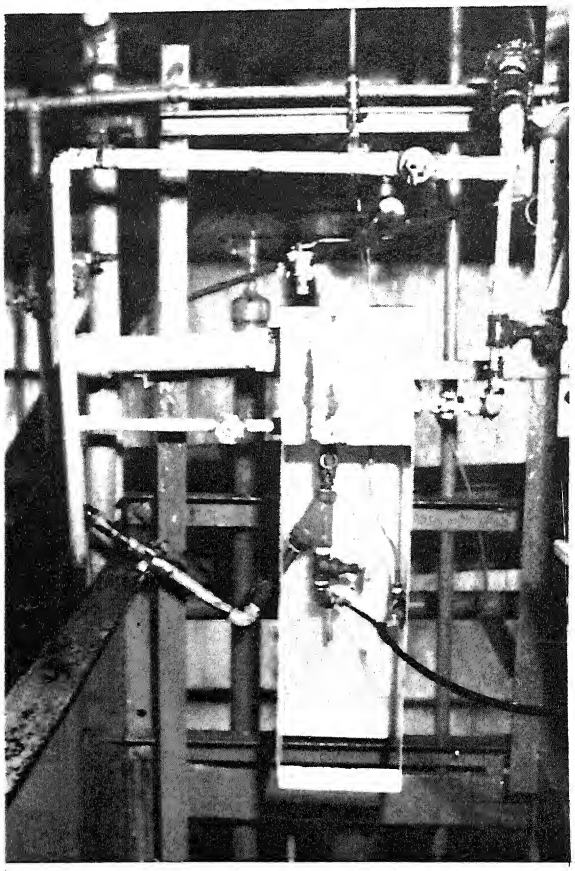

FIGURE 5--Sampling arrangement for washed raw sugar liquor in Montreal refinery.

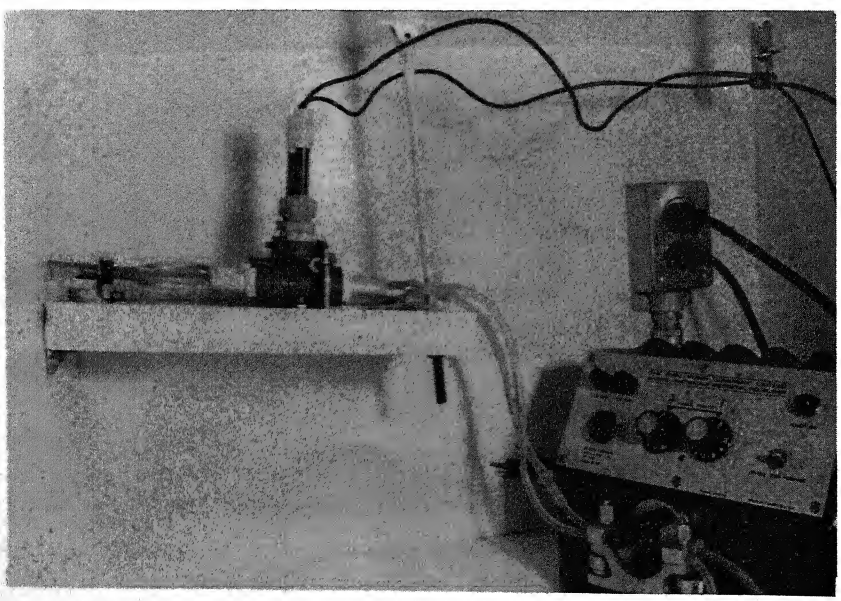

FIGURE 6--Chloride cells and pump in Montreal refinery.

Presence of Suspended Solids

The presence of very abrasive sand particles in the liquor is detrimental to the chloride electrode because of wear on the electrode sensing element. They also damage the tubing in the sampling system. It is essential to strain the liquor before it enters the sampling system.

Results

After several months of plant experimentation the system proved satisfactory. The last modification was to install, about two years ago, the digital millivolt readout and timer control in the control room. Figure 7 shows the compact installation for chloride measurement in the Montreal refinery, and the control room instrumentation is shown in figure 8.

A calibration chart has to be established every time a new pair of electrodes is installed. The calibration and the electrode checking can be done in the plant and the electrodes do not need to be taken to the laboratory. Life time of a pair of electrodes is about six months.

Figure 9 shows an actual calibration chart where chloride concentration is plotted against the millivolts reading. It is realized that the chloride concentration versus mV is a logarithmic relation but for all practical purposes, because of the narrow concentration range involved, we can assume a linear relationship. To confirm this, a regression analysis was run on the computer. Once the calibration has been done, an operating range for an acceptable chloride level in the washed raw sugar is defined as shown in figure 10, and the corresponding mV reading indicated to the operator, who manually adjusts the washing time accordingly in order to achieve the mV target. It should be noted here that the Montreal method is controlled based on a fixed target chloride in the washed raw sugar liquor independently of the chloride in the raw sugar. This approach, although different to Toronto's regression analysis method, is practical and experience has shown that the fixed chloride target is in the middle of the range of variation found in the Toronto refinery.

SIRUP BACK BOILING CONTROL

At this point, the investigations into chloride control have been completed in only one area of the plant other than affination, that is, in control of jet 1 back boiled to first pan boilings. Various new problems arose in the course of setting up electrode control for back boiling.

Problems in Back Boiling Control

It is felt to be essential that the liquor analysis of the various pan boilings should be as constant as possible. Therefore, any fluctuations in primary feed stocks, regardless of the cause of these fluctuations, are compensated by adjustments in the sirup distribution.

It is realized that the various ratios of impurities to chloride will not be constant but it is felt that chloride control is or can be a major tool in

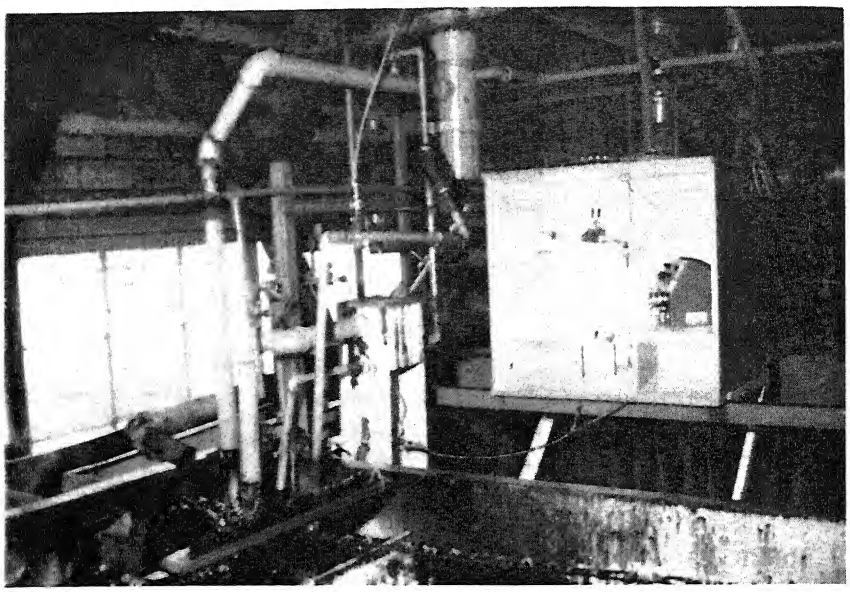

FIGURE 7--Chloride measurement installation in Montreal refinery.

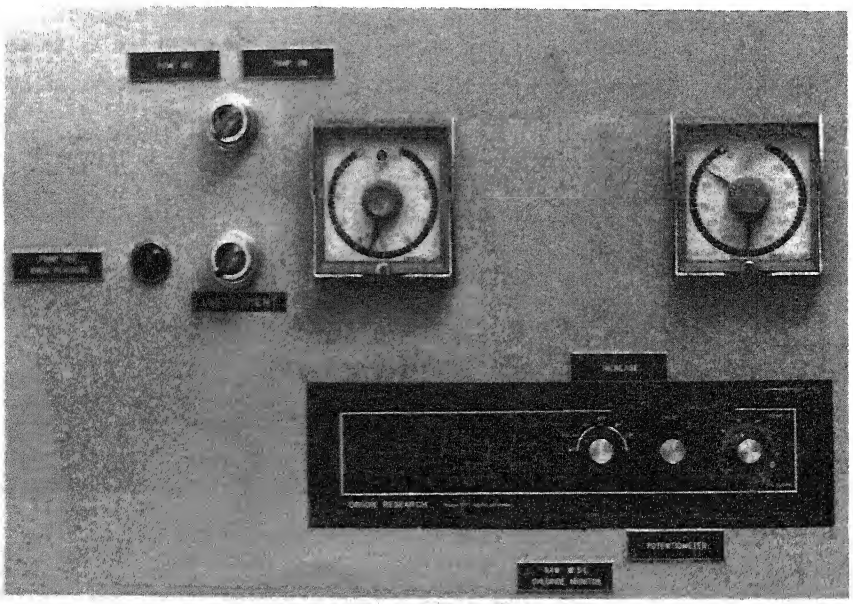

FIGURE 8--Control room instrumentation for chloride measurement in Montreal refinery.

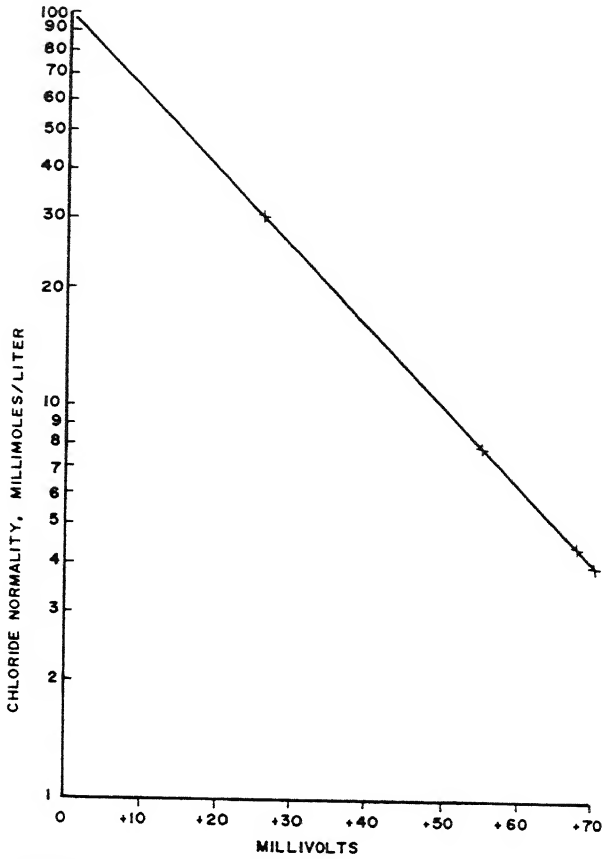

FIGURE 9--A calibration chart for chloride electrodes in washed raw sugar liquor.

implementing a process manager's day-to-day decisions. This is particularly true where his control emphasis may have to change due to raw sugar peculiarities and differences. As the impurities concentration in a massecuite increases, the subsequent sugar yield decreases. A refinery manager asks of his sugar boiler that he produces on a given day a particular MA sugar with as consistent CV as possible, and this goal is much easier to attain if the liquor to pans is fairly consistent with no sharp changes in impurities concentration. Our sugar boilers are provided with the very best in mechanical equipment, so it is, therefore, logical that they be provided with as consistent a feed stock as possible.

Fluctuations in fine liquor analysis are inevitable, and are generally compensated for by adjusting the amounts back boiled and by changing the sirup discards. Even though there is a continuous attempt to keep the first massecuite quality constant, there has been and always will be a delay in the response time of process personnel to liquor quality shifts, and this factor must also be considered in the development of a back boiling control process. The estimate of back boiling required is complicated by the fact that there is a marked simultaneous change in yield due to the change in the fine liquor analysis.

In the Toronto refinery, the fluctuations experienced in fine liquor ash are shown in table 2, together with the usual fluctuations in jet 1 analysis.

TABLE 2--Back boiling required to attain target ash in fine liquor shown as weight (lb) of jet 1 solids required per 100 lb fine liquor solids.

Target:0.11% ash

Fine liquor ash	Jet 1, % ash						
	.25	.26	.27	.29	.31	.33	.35
.06	35.71	33.33	31.25	27.77	25.00	22.72	20.83
.07	28.57	26.66	25.00	22.22	20.00	18.18	16.67
.08	21.43	20.00	18.75	16.67	15.00	13.64	12.50
.09	14.29	13.33	12.50	11.11	10.00	9.09	8.33

Target:0.12% ash

Fine liquor ash	Jet 1, % ash						
	.25	.26	.27	.29	.31	.33	.35
.06	46.15	42.86	40.00	35.29	31.58	28.57	26.09
.07	38.46	35.71	33.33	29.41	26.32	23.81	21.74
.08	30.77	28.57	26.67	23.53	21.05	19.05	17.39
.09	23.08	21.43	20.00	17.65	15.79	14.29	13.04

This table clearly shows the magnitude of jet 1 back boiling changes which should be made by refinery personnel as a direct result of changes in fine liquor, to achieve a resulting ash of either 0.11% or 0.12%. It is obvious from this table that it is difficult for process personnel to anticipate the magnitude of the changes in back boiling required to adjust for a change in fine liquor analysis from 0.06% ash to 0.09% ash. This may explain in large part why the refinery's jet 1 analysis has such a wide spread.

The system for boiling white sugar at Toronto refinery is a "3 and a bit" system, as outlined in figure 11. All fine liquor together with jet 1 back boiled is accumulated in a pan supply tank (tank 600 in figure 11) and may be drawn into either "A" or "B" pan for first strike boilings. Second and third strikes may follow firsts in the same pan, or vice versa.

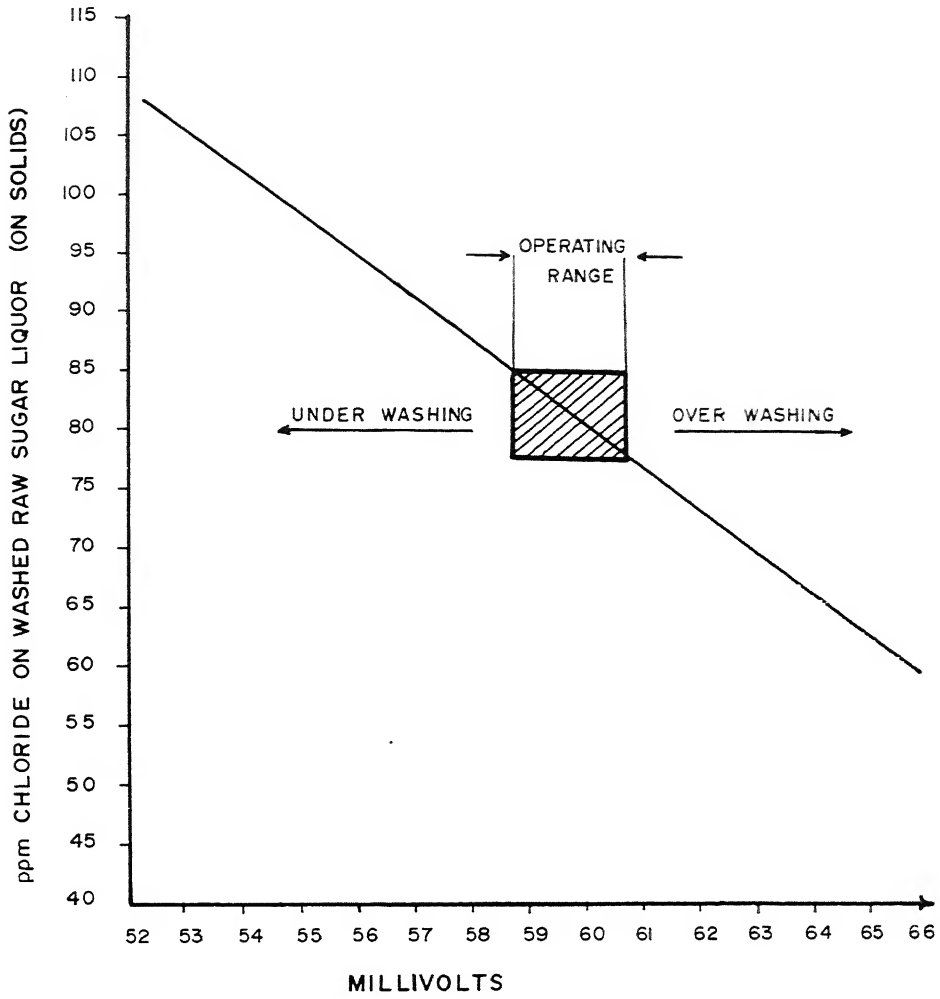

FIGURE 10--Calibration chart for chloride electrodes converted to show operating range.

Chloride Monitor for Back Boiling Control

Investigation on the chloride monitoring system was initially set to determine the sensitivity and reproducibility of an electrode measurement in an on-stream environment measuring chloride in jet 1. Laboratory evaluation had indicated that ± 3% error might be expected. To determine the error on-stream, a regression analysis technique was used.

Jet 1 was monitored with a chloride electrode connected to a strip recorder, and manual samples were taken and analyzed as shown in figure 12. The analytical results were compared to the mV strip recording. From this data a computer regression analysis was done, and a "best fit" curve derived. Figure 13 shows an actual strip chart record, and figure 14 the regression analysis of these results. In a system such as Toronto's, inadvertent mixing of jets is possible either through mass in the pan receiver or through incorrect operation of the sirup diversion systems. All possible steps to prevent this mixing have been

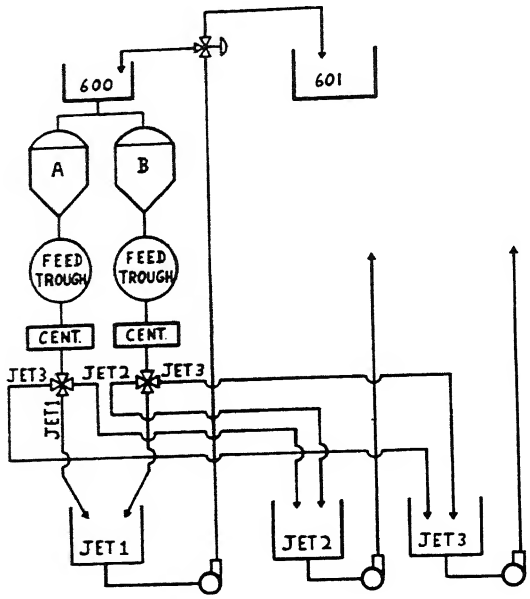

FIGURE 11--White sugar boiling system at Toronto refinery.

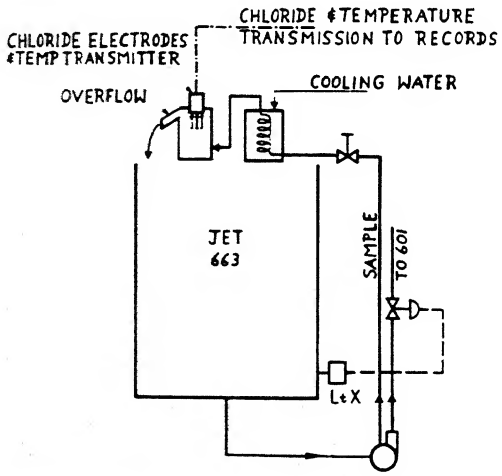

FIGURE 12--Monitoring of jet 1 with chloride electrode and sampling for laboratory determination of chloride.

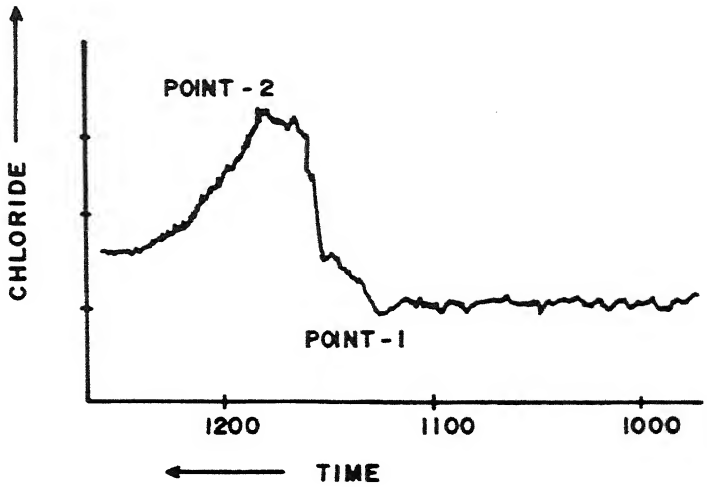

FIGURE 13--Strip chart record of chloride ion in jet 1.

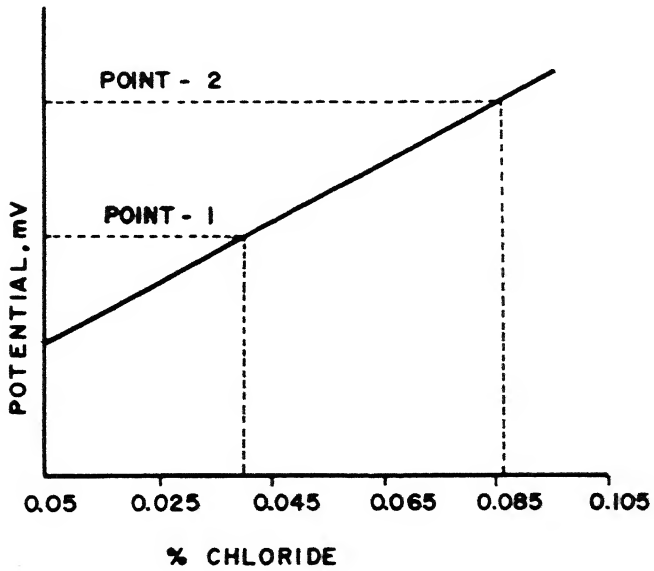

FIGURE 14--Regression analysis on laboratory chloride determinations and chloride electrode readings determined in process.

taken in the Toronto refinery through the use of enunciator alarm panels as well as employee education. Process personnel had been convinced that no mixing was occurring, until the chloride electrode began monitoring jet 1 on-strea and the evidence of mixing was clear.

In figure 13, points 1 and 2 show clearly that at the time of recording, jet 3 was being introduced into the system. This record allows a quantitative evaluation: jet 3 chloride was 0.254% on solids.

The recording strip chart stability presented convincing evidence of the value of this type of control on this station and it has been decided to install such a system, eventually marrying it directly to the computer for the purpose of ongoing solids distribution and pan yields analysis.

SWEETENING OFF CONTROL

We have data on this application of the chloride electrode but we are not prepared to present these at this time. However, we have plotted curves for this application and it does look very promising, particularly when the data is sent directly to the computer.

SUMMARY

In summary, it has been found that chloride is a highly satisfactory control in affination on either washed raw sugar or washed raw sugar liquor, and in back boiling the first sirups. The chloride electrode is stable, reliable and practical. The system is adaptable to remote control, automation and on-line computer control, and saves a great deal of control laboratory work.

DISCUSSION

H. M. WALLENSTEIN (CPC International): I am interested in the control you have achieved at the Montreal refinery on the affination station. Since you are taking your sample from the washed raw sugar melter, are you controlling the timing on the whole battery of centrifugals?

C. R. BROWN: Yes, we adjust the wash on all of the machines.

H. M. WALLENSTEIN: Do you find much interference here? Does much calculation have to be done to take into account the effect of the melting water?

C. R. BROWN: At one time, we were running into a problem with the chloride in the melter water, but at that time we were trying in the Montreal refinery for a very precise form of control. We took away a couple of the high Brix ranges of the sweetwater and used it for something other than melting and that solved the problem. Yes, there can be a problem, but we have achieved a steady condition now.

H. M. WALLENSTEIN: Do you find that the equipment calibration requires much attention?

C. R. BROWN: No. The calibration is done in the plant. Essentially what we are doing is a quality control idea: one of our fellows in the laboratory goes up to the electrode station once a day, takes a sample, brings it back to the laboratory for titration, and checks that we are getting the real chloride value from the electrode. Before we started filtering the liquor, there were quite a lot of problems, but we found that the suspended solids were wearing

away the electrode, and so avoided that problem by filtering the solution before it is tested.

H. R. PRIESTER (Savannah Foods): Do you think that chloride and ash measures are equally good controls for all raw sugars, or does one or the other method work better for some raws?

C. R. BROWN: The chloride control can be a particularly useful tool for a process manager or process operator when he is faced with the situation where he has to change emphasis. I think that chloride is the ultimate method of control; for example, if you are accustomed to controlling on ash, you can take a sample, do an ash on it, find the chloride-ash ratio, and carry on with the same emphasis. If invert is a problem, you make the corresponding calculation, but use chloride as the monitoring system. This is what we do at the Toronto refinery, where we normally control invert, but we also have some raws coming in where we have to watch the ash and to control on that instead. I prefer the chloride technique because it is a continuous monitor. You don't have to have laboratory personnel making hourly measurements.

In the Toronto refinery, as you saw in table 1, we feel that chloride is the best way to monitor the affination station simply because there is a much greater degree of removal of chloride, with a regression-based analysis of the wash. If you fluctuate the wash, and measure the ash, the invert and the chloride, you will find a more consistent removal of chloride than of either invert or ash. We have found this for many, many raw sugars. So as far as we are concerned, chloride is the way to control.

R. CORMIER (Redpath Sugars): What would be the cost of equipment, without a computer?

C. R. BROWN: There are four different systems available. The Orion Company[1] sells a full package for about $5,000. The package that we put together in our own plant, using our own voltmeters, etc., costs about $1,200; that includes the pump and other equipment, but not the control valves in the system. The chloride electrodes are about $250 each; voltmeters are about $500 each.

M. A. CLARKE (C.S.R.R.P.): You mentioned a calibration with each new electrode, about every six months. Do you have to calibrate between changing electrodes?

C. R. BROWN: There can be a shift in the absolute reading of the electrode from day to day. At one time in Montreal refinery we calibrated once a week or so, but we have found that that is not necessary. Now we take a daily sample and make sure that there is no drift from day to day; that is, we check the intercept of the calibration curve. Once we solved the problem of the membrane's being worn away by solid particles abrasion, we found that the electrodes were a lot more stable than we thought they were going to be.

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An additional point here about reference electrodes: in his studies on those, Dr. Pommez has found that the use of the double junction reference electrode and combination single electrodes has been found to be totally unreliable for our purposes.

M. MATIC (Sugar Milling Research Institute): I understand that you take a reading every three or four minutes, when you get stable conditions. What then is the purpose of this continuous monitoring--couldn't you take a sample every five minutes and do it by chloride titrator? What is the purpose of having a continuous process if it is not doing a continuous job?

C. R. BROWN: Yes, what you are suggesting is one way of doing chloride control. However, you have to have somebody take the sample and do the titration, and that requires additional personnel. In our case, we have a single control operator, in a control room, covering a wide part of the plant. He has only to look at the chloride meter, and make an adjustment. The site of the chloride meter is remote from the control room, and the operator performs other functions as well as monitoring the chloride analysis. In addition, if you consider the automatic regression-analysis technique, the equivalent manual calculations would require three or four hours.

FLUORESCENCE MEASUREMENTS AND pH SENSITIVITY AS PREDICTORS OF COLOR REMOVAL IN PROCESS

By Donald F. Charles[1]

ABSTRACT

Procedures were developed to quantitate two major types of fluorescence. Comparisons were made of removal of fluorescence and of pH 4, 7, and 9 colors in clarification, bone char and granular carbon adsorption, and crystallization. Fluorescence did not contribute significantly beyond pH sensitivity in predicting pH 7 color removal. Regression equations were calculated, aimed at predicting granulated pH 7 color from raw sugar crystal color parameters; raw crystal color at pH 4 was most significant with a small additional contribution from raw crystal color at pH 9.

INTRODUCTION

For several years C and H has been interested in the fluorescence exhibited by sugar solutions. It was recognized that fluorescence might be related to color as normally read at 420 nm wavelength. An Aminco-Bowman Spectrophotofluorometer and an x-y recorder were set up to permit automatic plotting of fluorescence intensity as wavelength was scanned. Either excitation wavelength can be preset and emission wavelength scanned, or emission wavelength can be preset and excitation wavelength scanned. Figure 1 shows the type of plots obtained for "A" fluorescence in sugar solution using both methods.

With C and H granulated sugars, two major fluorescence peaks seemed to appear consistently throughout the refining operations. Their characteristics are listed in table 1. Because the wavelength of excitation for "A" fluorescence and wavelength of emission for "B" fluorescence were near the wavelength 420 used in color measurement it seemed especially pertinent to study these two peaks.

TABLE 1--Characteristics of fluorescence types

Type	Wavelengths (nm)		pH Sensitive
	Excitation	Emission	
A	395	515	yes
B	320	410	no
QS	345	445	---

QS is quinine sulfate, the intensity standard.

[1]Research chemist, California and Hawaiian Sugar Company, Crockett, Calif. 94525.

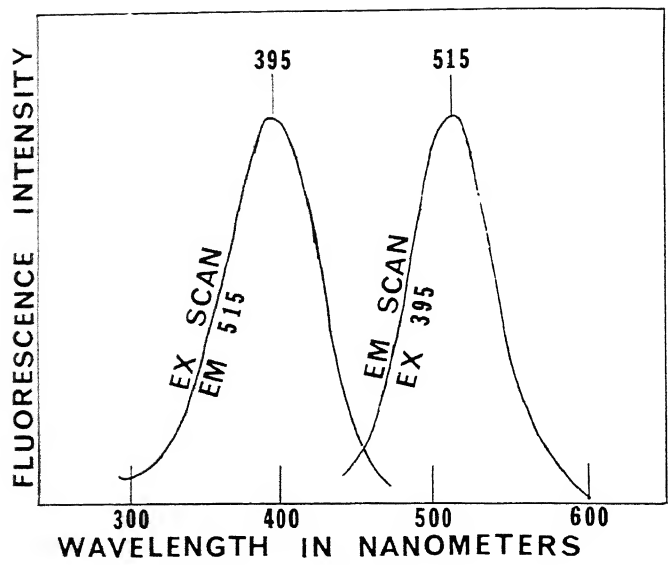

FIGURE 1--Excitation and emission peaks of the "A" type fluorescence in sugar solution.

For dark process liquids the maxima of excitation and emission appeared at somewhat higher wavelengths than those listed in table 1. On dilution, the maxima shifted to lower wavelengths. It appeared that absorption of exciting light was a major factor in the wavelength of the maxima. Absorbance at pH 9 decreased rapidly with increasing wavelength in the portion of the spectrum between 380 and 420, especially in sugars with a high value of pH sensitive color.

It was planned to make quantitative measures of "A" and "B" fluorescence as well as sensitivity of color to pH and to try to decide which factors were the best indicators of pH 7 color removal through each processing step studied.

This paper will discuss primarily the fluorescence effects, since pH sensitivity effects were reported on previously by Norman Smith[2] of C and H.

ANALYTICAL PROCEDURES

Solutions were prepared in concentrations of 50 Brix for granulated sugars, and 20 to 30 Brix for less pure samples. A refractometer reading established

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[2]Smith, N. H. 1964. The pH sensitivity of sugar colors and ease of decolorization. Proc. Tech. Sess. Cane Sugar Refin. Res. 1964: 14-25.

the g/ml solids to permit calculating ratio of intensity to solids. Into a 100 ml volumetric flask was pipetted a chosen volume of the diluted sugar solution of known Brix, plus 50 ml of ammonium acetate buffer of pH 4, 7, or 9, and water added to volume. Absorbance was read, on all solutions, and the "A" and "B" fluorescence was read on the pH 9 dilutions. The instrumental response was standardized by adjusting to give a fixed reading on a quinine sulfate standard solution. As table 1 shows, the wavelengths of maximum excitation and emission for the quinine sulfate were between those of the "A" and "B" fluorescence.

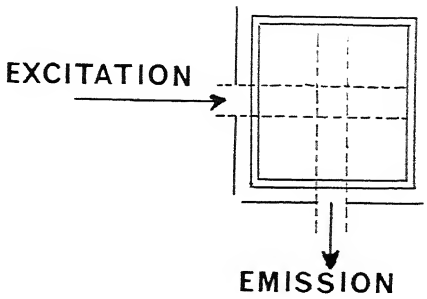

FIGURE 2--A horizontal cross section of the sample cell.

Laboratory work using different dilutions suggested the necessity of making corrections for absorbance. Figure 2 shows a horizontal cross-section of the sample cell. A slit width of 2 mm limited the width of excitation and emission light paths. As figure 2 illustrates, each beam traverses approximately one-half the cell. Since color readings at 420 nm were part of the study, it appeared possible to use the 420 nm absorbance at pH 9 as a correction to the "A" fluorescence, and this seemed to work out.

The formula for correction is:

$$\begin{matrix}\text{corrected} \\ \text{fluorescence} \\ \text{intensity}\end{matrix} = \frac{\text{observed fluorescence intensity}}{T},$$

where T = fractional transmittance,

A/cm = absorbance per centimeter,

and $T = 10^{\left(\frac{-A/cm}{2}\right)} = \text{antilog}\left(\frac{-A/cm}{2}\right)$.

As the formula indicates, % T can be calculated from half the corresponding absorbance; then % T is divided into the observed fluorescence intensity to get corrected fluorescence intensity.

"A" fluorescence is corrected for pH 9 420 nm absorbance, and "B" fluorescence for both 320 nm and 420 nm absorbance at pH 9. A program was developed for a Wang 600-6 computer that calculated, from a series of test readings, the corrected fluorescence intensity for each of a series of different dilutions of the same starting solution. The corrections used were shown to be both necessary and very nearly sufficient to give equal results for all dilutions.

Optimum dilutions were chosen for each class of product to give A/cm < 0.2 to minimize these absorbance effects when making fluorescence readings. Corrected values for "A" and "B" fluorescence are shown in Appendix.

RESULTS

Our standard procedure for studying raw sugars is to do a laboratory affination, then a defecation-filtration, then to pass the liquor over a char column, and finally to crystallize. The effect of each of these laboratory steps on the levels of "A" and "B" fluorescence will be discussed.

Clarification

A major processing step is the phosphate and lime defecation-flotation process, carried out in flotation clarifiers.

Table 2 shows analyses of the several parameters for two tests on liquor going to and coming from the process clarifiers. The table shows the change in each parameter on passing through the clarifier step, both in the basic ICUMSA units and as a percent of the on-liquor value.

The clarifiers seem to have little effect on "A" or "B" fluorescence as indicated by the small percent changes shown in table 2. The percent change is also small for 9-7 color, the parameter which is most significant in expressing pH sensitivity. On the other hand, clarification removes about 20% to 30% of the color as measured at pH 4 or pH 7. The 7-4 parameter decreases by about the same amount. Thus it appears that clarification removes primarily pH-insensitive color.

These relations can be considered as color ratios. The 9/4 and 9/7 increase through the clarifiers whereas the 7/4 shows little change. The off-liquor is more pH sensitive because of the removal of pH-insensitive colorant.

Additional work has suggested that the clarifiers remove primarily high molecular weight color and colloidal or suspended materials. The high molecular weight colorant fraction obtained by gel permeation methods turns out to be almost pH-insensitive. A rather high correlation might be expected between pH 4 color and quantity of high molecular weight colorant in raw liquors.

Bone Char

Figure 3 illustrates the trends of change in color and fluorescence with filter age for a laboratory bone char column. The column was 110 cm high, 2.4 cm diameter; volume flow rate was 4.0 ml/min, and void volume 160 ml. The pH sensitive color as measured by the quantity 9-7 is removed better than pH 7

TABLE 2--Removal of parameters in clarifier process (ICUMSA units)

	420 colors and differences				
	9	9-7	7	7-4	4
On	2944	1815	1115	409	706
Off	2855	1770	906	334	572
Off-on	-255	-45	-209	-75	-134
As % of on	-8.7	-2.5	-18.8	-18.3	-19.0
On	3317	1841	1476	530	947
Off	2811	1785	1026	350	676
Off-on	-506	-56	-450	-180	-271
As % of on	-15.2	-3.0	-30.5	-34.0	-28.6

	Color ratios			Fluorescence	
	9/4	9/7	7/4	A	B
On	4.18	2.64	1.58	686	138
Off	4.69	3.15	1.58	670	142
Off-on	+0.51	+0.51	0.00	-16	+4
As % of on	+12.2	+19.3	0.0	-2.3	+2.9
On	3.51	2.25	1.56	653	162.5
Off	4.16	2.74	1.52	653	151.5
Off-on	+0.65	+0.49	-0.04	0	-11.0
As % of on	+18.5	+21.8	-2.6	0.0	-6.8

color at the beginning of the run and less than pH 7 color later in the run. "A" fluorescence is also removed well in the early stages compared to later stages. Apparently the capacity of the char for these pH sensitive substances is low. The portion that adsorbs these substances is probably mainly carbon; it becomes saturated faster than the portion which works on the pH insensitive color.

"B" fluorescence is the most difficult group to remove with bone char.

Granular Carbon

Figure 4 illustrates the analysis of effluent liquors from process granular carbon columns as percent of the parameter in the liquor on to carbon. Here, it can be seen that the pH sensitive components (9-7 color) and especially "A" fluorescence are much better removed by granular carbon than is pH 7 color. "B" fluorescence is only a little harder to remove than pH 7 color.

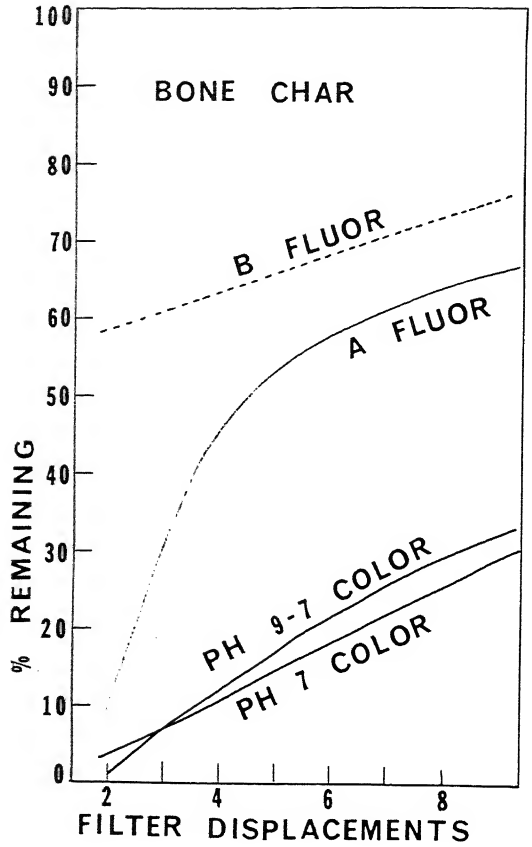

FIGURE 3--Removal of color and fluorescence with age of bone char.

Crystallization

Table 3 shows the proportion for each of the five parameters which boiled into the crystal. Observe that pH sensitive color expressed either as the 9-7 parameter or the 7-4 parameter tends to boil into the grain in larger proportion than the pH 7 color. "A" fluorescence especially tends to appear in the crystal. "B" fluorescence shows the lowest fraction appearing in the crystal.

Figure 5 shows "A" fluorescence in the crystal as it varies with "A" fluorescence in the liquor. A straight line through the origin with slope 0.45 fits the data, indicating that "A" fluorescence boils into the crystal in proportion to the amount in the liquor; that is, about 45% of that in liquor appears in crystal. Thus "A" fluorescence, which is very pH sensitive boils into the crystal more than does any of the other four parameters.

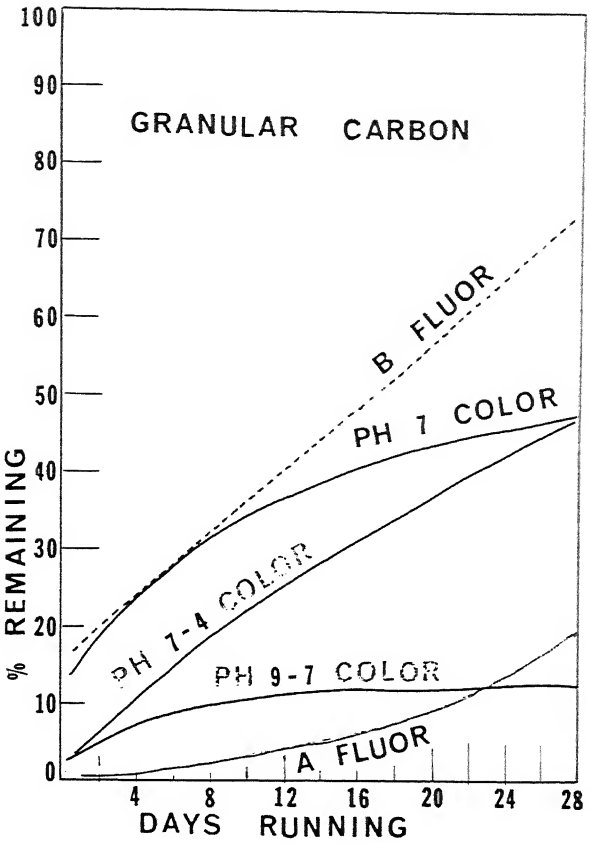

FIGURE 4--Removal of color and fluorescence with age of granular carbon.

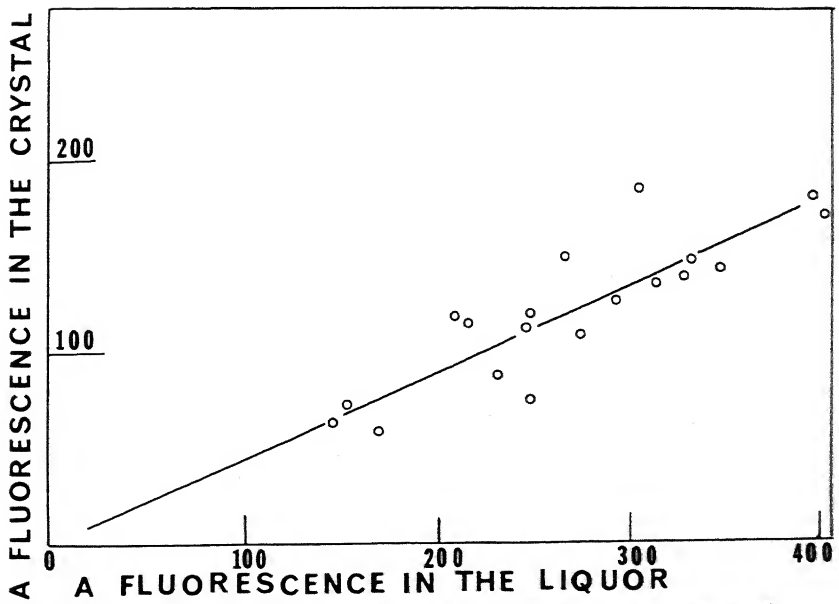

FIGURE 5--Variation of "A" fluorescence in crystal with "A" fluorescence in liquor.

TABLE 3--Quantity in crystal as percent of quantity in liquor

Parameter	Percent
pH 7 color	18
pH (9-7) color	28
pH (7-4) color	26
A fluorescence	45
B fluorescence	13

In figure 6, however. it appears that the amount of "B" fluorescence appearing in the crystal depends very slightly on the amount in the liquor, and varies little over the whole range. The average percent in crystal on liquor is about 7.0/60 x 100 = 12%. Thus "B" fluorescence, which is not pH sensitive, boils into the crystal less than does any of the other four parameters.

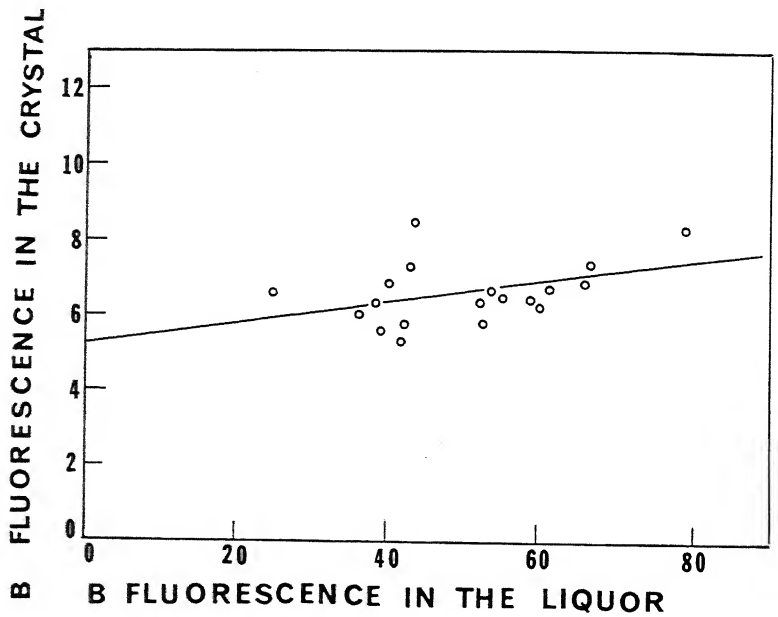

FIGURE 6--Variation of "B" fluorescence in crystal with "B" fluorescence in liquor.

This contrast between the behaviors of "A" and "B" fluorescence is of interest: it would appear that the "A" fluorescence, which is very pH sensitive, is equilibrium dependent, thus suggesting relatively strong adsorption effects. The "B" fluorescence, however, is incorporated in the crystal in a more or less fixed amount suggesting simple inclusion in proportion to crystal volume.

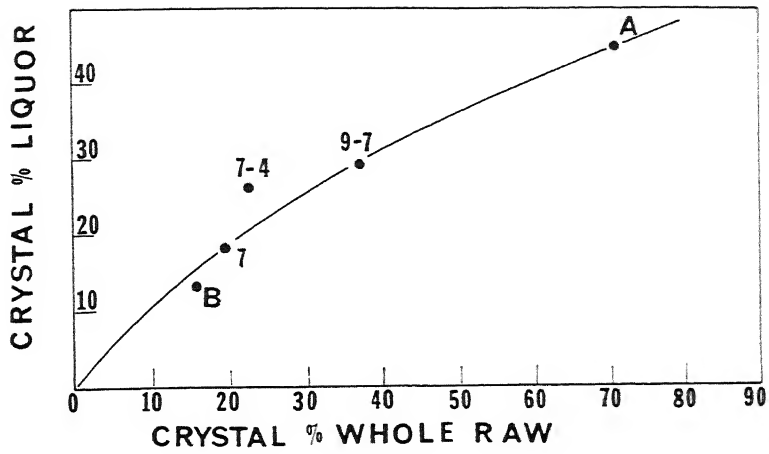

FIGURE 7--Proportion of parameters in crystal comparing granulated against raw.

Measurements of the parameters were compared in whole raw sugar and laboratory washed raw crystal. Figure 7 shows that the effect of crystallization is much alike for raw sugar crystallization and for number one liquor crystallization. The vertical scale is the quantity in the crystal as a percent of number one liquor, as taken from table 3. The horizontal scale is the quantity in crystal as a percent of whole raw sugar quantity. There is a fair correlation: "B" fluorescence is low in both crystals; "A" fluorescence is high in both cases. The pH sensitive color group also tends to boil into the crystal compared with the average pH 7 color. The 7-4 color seems to be most out of line. This may suggest a different character to the 7-4 color where it is going from raw to granulated.

From these data were developed regression equations as models of the effect of concentration of on-liquor parameters on pH 7 color in liquors leaving the major process steps. In all cases we found that pH sensitivity was a better predictor than "A" or "B" fluorescence, i.e., that behavior of the pH sensitive colorants best predicted liquor color at pH 7. For char decolorization, however, the pH 4 color was the best predictor of pH 7 color in liquor off char. For crystallization, pH 7 color was the best predictor, but pH 9 color added a significant amount to pH 7 color; i.e., high pH sensitive color tends to boil into the grain and make high pH 7 color.

Equations were developed to predict the granulated crystal color from color parameters of the whole raw sugar and washed raw crystal. From 33 data sets table 4 was derived. Here it can be seen that pH 4 color is a better prediction criterion than pH 7 color, and that pH 9 color adds very little. There is apparently a degree to which the processing steps are complementary and the significance of each is reduced somewhat when the overall process is considered.

TABLE 4--Overall process-predicting ability of equations

Independent parameters in regression	% of variation in granulated pH 7 color explained	
	Total	Added
Only crystal pH 7 color	50.6	50.6
Only crystal pH 4 color	56.1	5.5
Add crystal pH 9 color	58.5	2.4
Add crystal pH 7 color	58.9	0.4

The conclusion is that pH 4 color can explain about 56% of the pH 7 color in granulated sugar. pH sensitivity explains only another 2.8%. "A" fluorescence and "B" fluorescence have no significance relative to the pH 4, 7, and 9 colors.

SUMMARY

Two major types of fluorescence have appeared in raw sugars that we have examined.

Table 1 lists important characteristics of the fluors, labeled "A" and "B", along with data for quinine sulfate, QS, which serves as an intensity standard.

We measured removal of the fluorescence and pH sensitivity parameters in major processing steps. Clarifiers removed primarily pH insensitive colorant. Bone char initially removed pH sensitive components but on average did the best removal on the basic pH-insensitive colorant. Granular carbon removed "A" fluorescence best, pH sensitive color next best. Crystallization removed "B" fluorescence best, and "A" fluorescence the least; pH sensitive color also boiled in.

pH sensitivity was always a better predictor of pH 7 color removed than fluorescence. For the overall process, to predict granulated pH 7 color, raw crystal pH 4 color was best with a small additional contribution from raw crystal pH 9 color.

Appendix--Calculations for fluorescence corrections

1. Corrections on "A" fluorescence for absorbance at 420 at pH 9.

Dilution factor	Raw "A" fluorescence	X dilution factor	Corrected for 420
2	1560	3120	648.8
2.25	1440	3243	639.6
3.01	1230	3697	659.0
4	1050	4200	699.4
5	860	4300	680.0
10	490	4900	711.5
20	246	4920	683.7
C.V.	49.3	18.0	3.93

2. Corrections on "B" fluorescence for absorbance at 420 and 320 at pH 9.

Raw "B" fluorescence	X dilution factor	Corrected for 320	Corrected for 420
117	234	96.9	152.1
123	277	101.6	151.3
126	379	109.5	147.3
120	480	114.6	144.1
111	555	117.6	140.4
79	790	134.0	146.9
49	980	147.0	154.3
C.V. 27.8	51.8	15.1	3.29

DISCUSSION

E. OBST (Amstar): Are you absolutely sure that the fluorescence comes from the sugar, and that it is not from the solvent or some reagent? I have been using distilled water as a standard, and sometimes even that can have some fluorescence.

D. F. CHARLES: We didn't continually check everything as a routine thing. In the early stages of the work, we did look at our distilled water--actually we used distilled and de-ionized water to dissolve the sugars--and we found that there wasn't any obvious fluorescence at least at the points we were studying. There might have been some very small peaks less than a thousandth the intensity of those we were observing. We were pretty sure that this fluorescence was from the sugar. For the first few times, when we set up the instrumentation, we would actually zero with water also. Routinely, however, one makes zero and dark current adjustments with the shutters closed.

E. OBST: This area certainly needs to be developed. The research is very interesting.

F. G. CARPENTER (C.S.R.R.P.): Are these fluorescent materials colored or colorless?

D. F. CHARLES: This is an interesting question. We really haven't determined the amounts of materials present, and the color depends on that too. When you measure a fluorescence intensity, you don't know its quantum output or how much of the compound is there. In the sense that excitation at 395 nm, as for "A" fluorescence, represents a certain amount of absorbance at 395 nm, which is very near the wavelength 420, then you must say there is some color there.

F. G. CARPENTER: I want to point out that these compounds are absorbing, and absorbance means color. They absorb in the ultraviolet, so you don't see where they absorb, but you do see where they fluoresce. It is possible to have a very white sugar that gives a very clear solution with little color but a high fluorescence, so there is not 100% correlation between color and fluorescence.

D. F. CHARLES: No, certainly not 100% correlation. One of our main questions is, how much relationship is there? If we define color as absorbance in the visual spectrum, we would have to say that "A" fluorescence is not necessarily color, since its absorbance at 320 nm is well into the ultraviolet.

F. G. CARPENTER: In your B fluorescence, it appeared that the amount in the crystal did not depend on the amount in solution. Could it be that the fluorescing compound was being manufactured in crystallization, so that its quantity in the crystal depended on the amount of sugar there rather than on the amount of impurity in solution?

D. F. CHARLES: That is a possibility. However, nothing in our experience so far suggests that this happens. We could make some tests to study this idea. We do have reason to think that some changes occur, but more likely in the earlier stages of the process, particularly in the raw sugar mill. We might get formation or destruction of a fluorescent substance. There has been some work showing that fluorescent substances are produced in processing.

R. CORMIER (Redpath): With regard to your A fluorescence, measured at basic pH, I think that even traces of glucose and fructose at basic pH might give some enolate ions in solution. This might be easily confirmed by

absorption spectrophotometry. I don't know the exact excitation wavelength of enolate ions, but perhaps they could be responsible for your fluorescence. These compounds are not colored, but might give colored compounds after transformation to ring aldehydes or other products.

D. F. CHARLES: You are suggesting that the amount of A fluorescence might be correlated with the amount of invert sugar present.
(BY CORRESPONDENCE) On review, it appears that my correlation with invert level is tenuous. We have observed raw sugars to differ by a factor of 20 in "A" fluorescence intensity when invert level is almost equal.

R. CORMIER: At pH 9, did you make some correlation between fluorescence and time? For example, were you getting one base or two?

D. F. CHARLES: Over a few hours or a few days, under refrigeration, we haven't seen any marked changes. It is possible though that some changes could occur, even in raw sugar or washed raw sugar sitting on the shelf. We found some differences, as I mentioned, between samples that we ran routinely for crystal color compared to samples prepared in the laboratory. We found some discrepancies there, where the results didn't agree. Maybe something was happening to the samples, even sitting on the shelf.

R. CORMIER: Investigations have shown that the time is something like up to 20 days before color forming products appear.

D. F. CHARLES: It is a question which could receive further study, but we have not emphasized this problem in our studies so far. We have not observed any obvious changes on storage.

COLORANT FORMATION UNDER REFINING CONDITIONS

By F. G. Carpenter[1] and E. J. Roberts[2]

ABSTRACT

One type of colorant in commercial sugars is that formed in the course of refining processes. The literature on this subject is reviewed and current experimental work under controlled conditions is described.

INTRODUCTION

Although color removal is the primary purpose in refining sugar, it must be recognized that sucrose, like all organic compounds except methane, is unstable: even while color is being removed, more is being formed. The fact that color is formed in processing is well known, and is the reason that vacuum pans are used to boil the sugar below atmospheric pressure, at a low temperature where less color is formed. However, the details of the reaction mechanisms and the identity of the colored reaction products are not known at all. If these facts were known, they would provide a rational basis for deciding whether to remove the colorant formed or process to prevent its formation in the first place, to do both, or to do neither. In order to obtain useful information about these important questions, it is necessary to record carefully all details of the conditions of color formation, and to separate and characterize the colorants formed. These data will lead to the identity of the colorant and the nature of the formation reactions.

This study summarizes recent work and outlines the current status on the subject of colorant formation from sucrose. Consideration is limited to approximate refining conditions; that is, temperatures above 100°C are not considered, nor are very low or very high pH values, nor are very dark colored liquors, such as molasses. Rather, consideration is given to high Brix solutions and light colored components such as those first formed from the decomposition of sugar or the interactions of minor constituents. Also excluded are microbial or enzymatic reactions, which are only results of bad refining practice.

ACID REACTIONS

Sugar color formation can be systematized in several ways. There are the alkaline and the acid reactions, the reactions of sucrose, glucose, and fructose, and in particular the reactions of these sugars with amines.

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Considering the acid reactions, the first step is probably the inversion of sucrose, followed by further reaction of the reducing sugars which are well known to be inherently more reactive (or less stable) than sucrose. It has long been known that 5-hydroxymethyl-2-furfural (HMF) is one of the acid decomposition products of sugar (7) and that HMF in turn produces color. Neither the mechanism of formation of HMF nor that of the production of colorants from HMF is known, nor is the identity of the colorants produced from HMF.

Anet (2) has recently thrown some more light on the subject by showing that when a fructose solution is heated at low pH, two of the substances formed are the cis- and trans-isomers of 3,4-dideoxyglucosulose-3-ene (DGU) and that these are the immediate precursors to HMF.

It is also known (8) that although sucrose does not fluoresce, all commercial sugars show considerable fluorescence. Yoshihiro and Hashimoto (9) have reported that a green fluorescent substance (GFS) is quickly formed along with HMF when fructose is heated in solution at low pH. Burton, et al. (3) reported the development of fluorescence in glucose-glycine systems.

Since this laboratory has had considerable experience in both fluorescence and separation of colorants, this same sucrose degradation system was examined. A 50 Brix sugar solution was kept at 85°C for 5 days. If the pH was not acid initially, it soon became so. All solutions were at about pH 3 at the end of 5 days and all had developed some degree of color. The solution was then extracted with ethyl acetate to separate many of the colorants from the sugar. The extract was separated into components by high voltage paper electrophoresis, by methods which have been previously described (4).

Examination of the fluorescence of the resulting electrophorogram showed that in every case a bright green fluorescent spot predominated. This was undoubtedly the GFS of Yoshihiro and Hashimoto (9). Some DGU was made in this laboratory after the method of Anet (2), and by the usual chromatographic comparison methods were compared with GFS. After finding an exact match in three different chromatographic systems (2 thin layer and 1 high voltage paper electrophoresis), it could only be concluded that:

GFS of Yoshihiro and Hashimoto = DGU of Anet

The overall reaction involved is:

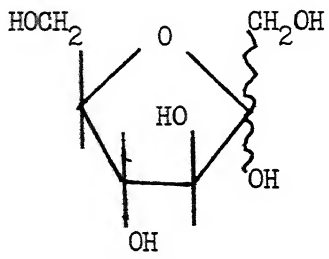
Sucrose
↓
Fructose
↓
GFS = DGU
↓
HMF
↓
Color

This scheme proved deceptively simple because 13 other spots were found along with the DGU. These were isolated, washed off the paper, subjected to

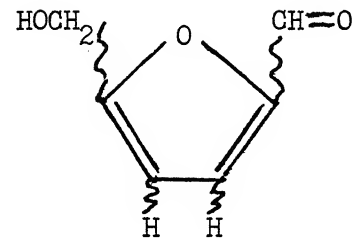
further heating and then to another electrophoresis. All 13 spot-forming compounds were reduced in content, and the DGU concentration was increased on the resulting electrophorogram. Therefore, it was concluded that all 13 compounds were steps in the production of DGU. This means that the step from fructose to DGU has at least 13 intermediates or alternate routes.

Details of the reaction have yet to be worked out and the reactions appear to be extremely complex.

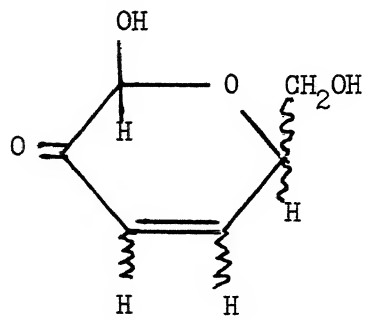
In looking at the structural formulae of fructose and HMF, one might be tempted to think of three water moieties splitting out of fructose to form HMF, but when the intermediate DGU that has been found is viewed between fructose and HMF, the simplicity of this mechanism fades away, and it is apparent that 13 intermediates could indeed be present.



FRUCTOSE



HMF



DGU

None of these compounds is colored but all fall into the category of color precursors. HMF has been measured by its UV absorption peak at 278 nm. However, this measurement is unreliable because many other compounds found in sugar absorb near this same wavelength. It would appear that the fluorescence of DGU might be a more sensitive measure of a color-labile sugar.

Further research into these various acid reactions might not be needed because the conditions causing the formation of this type of color have been found and several intermediates isolated. Presumably this type of color

formation could be prevented by avoiding the acid conditions or, if necessary, by finding a way to stabilize the intermediates or to redirect their reactions.

The solution, however, is not that simple because these same intermediates can be made by another route. Anet has shown (1) that a difructose glycine compound that is made by the Maillard reaction can rearrange to DGU, leaving the amine moiety free to repeat the reaction. This provides another source of the same intermediates and, therefore, the same colorants, by a different route. If there are two known routes, there are probably more, so it would appear to be worthwhile to continue this line of research.

ALKALINE REACTIONS

On the alkaline side, a whole new set of reactions takes place. These have been much studied by the beet sugar industry because their operation habitually runs at pH 9 or 10. Carbonatation clarification processes in cane factories and refineries also reach these pH values. There is a large body of literature on the alkaline degradation products of fructose (ADF). A great many intermediates have been identified and listed in an excellent recent review by Fleming, Parker, and Williams of Tate and Lyle (5). It is in the alkaline region where sugars easily isomerize and the reaction with amines proceeds well; yet, although this much is known, neither the identities of the colorant, the principal reactions, nor the intermediates have yet been established.

O'Donnell and Richards (6) have recently studied the initial steps in the alkaline degradation of sucrose, and in some excellent work have shown that the first step is a dehydration of the fructose moiety of sucrose. This is in contrast to the splitting of the fructose from the glucose in the acid region. One of the end products is lactic acid, but it should be noted that most of the lactic acid in commercial sugars comes from lactate-forming bacteria, to the point where lactate is used as a control for detecting bacterial contamination in some sugar installations. The rest of the steps leading to color are not understood, but it was felt that too often studies had been made under insufficiently well-defined conditions, particularly with regard to pH which always drops in the course of any sugar colorant-forming reaction. The lower pH changes the reaction to the more acid range reactions; the results have thus been confounded, and, instead of valid information, more confusion and controversial results have been obtained.

CONSTANT pH REACTIONS

One intriguing feature of the formation of sugar color is the apparent effect of various inorganic components on the color-forming reactions. However, it has not been shown whether the effect is from the inorganic component or the buffering action of the resulting salt system in maintaining pH.

Studies have, therefore, been made at a constant pH to eliminate one variable.

The pH-stat that this laboratory employed for these studies was simply an automatic titrator switched to its constant pH mode.

For this initial screening, fairly high concentrations of added substances were used. Ten mole percent of the substance was added to a 30 Brix sugar solution (1 molar). The solution was kept at 90±2°C for 16 h at a constant pH, with good stirring. NaOH was added to maintain the pH level. The color (in ICUMSA units) was measured at the operating pH before and after the treatment, and the difference reported as the color produced. The initial colors were all in the range of about 10 to 20 units.

TABLE 1--Effect of salts on color formation in 1 molar sugar solutions at constant pH

Salt 0.1 M	Color (ICUMSA units) gained in 16 h at 90°C			
pH	6.6	8.0	9.0	10.0
None	212	404	655	2000
$CaCl_2$	480	315	364	466
KCl	76	436	1500	1486
$BaCl_2$	---	236	350	200
$SrCl_2$	---	370	334	294
Calcium sucrate (alone)	170	178	229	300
KCl (KOH)	210	728	98	250
None (KOH)	58	178	38	95
$CaCl_2$ (KOH)	---	968	----	----
NaH_2PO_4	83	235	873	1400
Na_2HPO_4	---	210	----	----
Sodium citrate	0	2000	2000	5370

Constant pH maintained with NaOH unless otherwise stated.

For sucrose alone, the color development was higher at higher pH, as shown in table 1. With added $CaCl_2$ the alkaline color formation was much less, although color was a little greater in the acid range. To check whether it was calcium ion or chloride ion that was effective, the relatively non-pH changing salt KCl was added, and gave results opposite to $CaCl_2$; it thus appears that the cations were the active components.

Additional data with $BaCl_2$ and $SrCl_2$ more or less paralleled that of $CaCl_2$ and since barium ion and strontium ion are similar to calcium ion this tends to

reinforce observations of the effect of calcium. There is a way to add calcium ion alone, without other anions or the sodium ion of the NaOH used to keep the pH up. This is to use $Ca(OH)_2$ (in the form of calcium sucrate) to maintain the high pH. This does not, however, keep a constant calcium ion concentration. These results indicate that it is indeed the calcium ion that prevents color formation at high pH. Also, since less color was formed at low pH (where less calcium ion was present) it must be concluded that calcium ion causes color at low pH.

There still remains a question about the sodium ion that was used to keep the pH up. Runs were, therefore, made using KOH instead of NaOH. The results show that either potassium ion inhibits or sodium ion enhances color formation at high pH with the reverse effect at low pH, and a big increase in color with potassium ion at pH 8.0. This was confirmed with and without chloride and calcium ions present.

In other runs with sodium phosphate salts the color formation was similar to that observed with sodium alone at high pH, but the phosphate apparently inhibits color formation at low pH.

Spectacular results were obtained when a citrate salt was added. A complete inhibition of color at low pH gave way to a drastic enhancement of color at high pH.

Extraction of the constant pH solutions with ethyl acetate and examination of the extract with high voltage paper electrophoresis showed the expected results: alkaline solutions contained a quite different series of components than the acid solutions, none of which have been identified.

CONCLUSIONS

Under acid conditions the predominant color forming reactions in sugar solutions are through a degradation of sucrose pathway that includes fructose, DGU, and HMF, with many other intermediate compounds. Some of the same intermediates are made by the Maillard reaction involving amines and fructose. In alkaline solutions, the sugars easily isomerize and one of the first subsequent steps is the dehydration of fructose. The effect of various ionic constituents at constant pH is summarized as follows:

calcium ion inhibits color formation at high pH, enhances color formation at low pH,

sodium ion is the reverse of calcium ion,

potassium ion enhances color formation at pH 8 with less color formation at either extreme,

chloride ion has no effect,

phosphate inhibits color formation at low pH, and

citrate stops color formation at low pH but enhances it at high pH.

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DISCUSSION

W. FRIES (Rohm and Haas): We are in the business of evolving new resins to remove sugar color. I want to ask, generally, what advice you would give us in terms of testing our resins, and specifically, would molasses color be a sufficient and effective model for sugar colorant?

F. G. CARPENTER: I don't believe that molasses color should be used to test decolorization, because the sugar refiner does not decolorize molasses. The refinery decolorizes a fairly light raw sugar and the molasses, or concentrated color is the material that has been readily removed. The compounds in

molasses are the easy ones to get out. The colorants that must be taken out with ion exchange or adsorbents are not the ones that are dumped into the molasses. For resin test purposes, you are more interested in removal of the lighter colors that may remain in the grain; those are the ones which are more likely candidates for an adsorbent or an ion exchange color removal.

J. SULLIVAN (SuCrest): You measured color formed at low pH. Did you find measurable quantities of sucrose loss?

F. G. CARPENTER: We did not enter into this area, because the sucrose loss relationship to acid inversion is already well known. Several years ago, Athenstedt[1] at the Technischehochschule in Berlin published a very good paper on sucrose loss in the alkaline range. Incidentally, he showed that color formation proceeds much faster than sucrose loss. If you take both the curve for inversion rate and also the curve for alkaline degradation of sucrose, the minimum of the summation of these curves comes in the vicinity of pH 8. That minimum area is the place where sucrose is most stable in actual practice.

J. SULLIVAN: You said, I believe, that your treatment conditions to form DGU were 50 Brix, 85°C, for 5 days at pH 3.

F. G. CARPENTER: We did not initially set pH. Whatever sugar solution we started with, at whatever pH, ended up with a pH of 3 (or 2 in some cases) after 5 days at 85°C. A lot of color was formed in those solutions.

J. SULLIVAN: You mentioned that Anet in Australia has made similar compounds under similar conditions. Are you also going to look at the particular compounds obtained under refinery conditions?

F. G. CARPENTER: Yes. That green fluorescent substance, DGU, is one of the first ones to appear under acid conditions.

J. SULLIVAN: Do you think that substance is important to us in the refining process, or are you making colors that would not be found in a typical refinery?

F. G. CARPENTER: I think that these compounds are similar to those that Don Charles talked about, which are made in the refinery. There is a connection here between the fluorescent substances. His fluorescence "B" group did not seem to depend on the initial material, but appeared to me to be made from sucrose during crystallization.

J. SULLIVAN: I have a question related to past and current work of the project. Has anyone, anywhere, published recommendations for methods of removal of specific colorants, since quite a few have been identified now?

F. G. CARPENTER: At this time we have not done such a study and I don't know of anyone else who has done that. I expect if we found some particular

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[1]Athenstedt, Von M. 1961. Decomposition of sucrose in alkaline solution Z. Zuckerind. 11: 605-611; 661-668. Sugar Ind. Abstr. 24: 187.

compound that was responsible for a lot of problems, the adsorbent and ion exchange people would quickly find the best ways to remove it. I recall the problem, some years ago, at B. C. Sugars, where there was too much iron in the soft sugars. An ion exchanger was quickly made that just took out the iron.

C. C. CHOU (Amstar): Color formation is a very complicated system. It will take a long time, and extensive research, to identify the systems and compounds involved. Another approach is to study the population of a particular conjugated double bond, or particular functional group, which is present in the system and responsible for the color: then we can find ways to remove these color-forming groups.

F. G. CARPENTER: Dr. Wendell Binkley has done a great deal of work in this area, counting the number of double bonds and branch chains on that part of the color that goes into the molasses.

C. C. CHOU: To study the conjugated double bond, with a view to finding ways to remove their compounds, one could obtain emission spectra as a function of different excitation wavelengths. This would give you an idea of the nature and/or the population of conjugated double bonds.

F. G. CARPENTER: That is very good in theory, but in practice, there are apparently too many compounds mixed together to allow that kind of identification. Fluorescence is a good tool for confirmation, but not really for identification.

M. MATIC (Sugar Milling Research Institute): Do you know why citrate suppresses color formation?

M. A. CLARKE (C.S.R.R.P.): Citrate is an intermediate in the biological degradation (metabolism) of hexoses. We think that it redirects some of the pathways in the formation of color from fructose, and leads to the formation of noncolored products.

M. MATIC: You are keeping these solutions for 3 days at 85°C. and getting several compounds which are fluorescing.

F. G. CARPENTER: Yes, we found, in nearly all cases, 14 fluorescent intermediates from this acid treatment.

M. MATIC: Are you trying to pinpoint the first step in the reaction?

F. G. CARPENTER: That, or some other steps along the way that could be stopped.

M. MATIC: Could you do this by cutting back the time of the reaction? This would give you a much less complicated mixture.

F. G. CARPENTER: In practice, you don't keep a sugar solution in an acid pH as long as we did. We wanted to collect a lot of acid degradation products, and we were almost heading towards molasses, but we didn't go to molasses. That compound that gives that first green spot, however, forms within minutes.

M. MATIC: So that is one of the first formed? Or are there intermediates there in quantities too small to detect?

F. G. CARPENTER: When we eluted the other 13 intermediates off the paper, redissolved them in water, reheated them and put them back on electrophoresis, we found that a great deal more DGU had formed at the expense of these other intermediates. So the other compounds appear to be intermediates in DGU formation.

V. S. VELASCO (CPC International): How many compounds did you find in the alkaline range?

F. G. CARPENTER: There were a great many compounds.

V. S. VELASCO: Did you ever investigate a compound called metasaccharinic acid?

F. G. CARPENTER: No.

V. S. VELASCO: This is one compound that evidently forms in the alkaline range[2].

R. CORMIER (Redpath Sugars): May I propose that the effect of the metal ions on color formation might be due to complexation of the metal ions with the carbohydrate molecule, and that the protective effect might be related to the size of the ion. If you compare calcium ion and potassium ion, which are much the same size, with sodium ion, you can see the effect of this periodic ordering. It would also be interesting to look at the effect of ferric ions.

F. G. CARPENTER: I agree with you about complex formation with the metal ions; we have thought about that, but not worked on it yet. Dr. Parker, of Tate and Lyle, has done a good deal of work with ferric ions and color[3]. Evidently iron and polyphenolics complex to give very strongly colored compounds.

R. CORMIER: When you kept the pH constant, did you have a lot of sodium ions in solution--at pH 10, say?

F. G. CARPENTER: Yes, at high pH's there was a high concentration of sodium ion.

R. CORMIER: This, then, would further complicate the situation, because there could be competition between the cations for complex formation. The high concentration of sodium ions could erase the effect of the other ions.

M. A. CLARKE: To further check the ionic size factor, we could use lithium salts in this series of experiments.

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[2]Anet, E. F. L. J. 1965. 3-Deoxyglycosuloses (3-deoxyglycosones) and the degradation of carbohydrates. Ad. Carbohydr. Chem. 19: 181-218.

[3]Parker, K. J., and Williams, J. C. 1968. The isolation and properties of sugar colorants. Proc. Tech. Sess. Cane Sugar Refin. Res. 1969: 117-127.

TRACE CONSTITUENTS IN MOLASSES

By A. C. Morriss and W. M. Nicol[1]

(Presented by Margaret A. Clarke)

ABSTRACT

Analytical methods and legislative implications with reference to heavy metal contaminants and organochlorine residues in molasses are reviewed.

INTRODUCTION

Increasingly, foods and drugs safety authorities are reducing the maximum permitted limits of potentially harmful impurities, not only in foods for human consumption, but also in those used in the feeding of animals providing sources of dietary protein. Molasses, traditionally, has had the reputation of being a health food and a nutritious adjunct to animal diet, despite containing the accumulated nonsugar residues of sugar processing. There is a danger that, in the current welter of legislation designed to safeguard the consumer, molasses could appear in a very unfavorable light.

Two classes of impurity are being highlighted: agricultural chemical residues and the toxic heavy metals. This paper directs attention on the one hand to the problems associated with current or proposed legislation in different countries, should the sugar industry consider it necessary to take defensive action, and on the other hand to the need for improved and more efficient methods of analysis.

AGRICULTURAL CHEMICAL RESIDUES

The consideration of just one class of residue, the organochlorines, comprising such well known chemicals as DDT, aldrin, dieldrin and lindane will illustrate the complexity of analytical problems facing the industry. The organochlorines are very stable to hydrolysis and will withstand the rigors of temperature and chemical treatment associated with sugar processing. These impurities are such that they will be excluded from the sugar crystal and become concentrated in the molasses; more specifically, they will be found in blackstrap and beet molasses rather than refinery molasses. There is no simple analytical technique for the determination of organochlorine pesticides, and differentiation between the individual pesticides is even more difficult.

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[1] Research chemist and group leader, Tate and Lyle Ltd., Group Research and Development, Philip Lyle Memorial Research Laboratory, University of Reading, P. O. Box 68, Reading, Berkshire, England, RG6 2BX.

General Principles of Chlorinated Pesticide Determination in Molasses

Although there is extensive literature concerning the determination of low levels of pesticide in foods for human consumption (milk, eggs, vegetables etc.) no reference has been found specifically for the determination of pesticide residues in molasses, or any other animal feedstuffs. The general procedure for any sample follows below.

Selection of a Truly Representative Sample

This is often difficult with molasses, since a bulk sample could consist of fractions from many different origins, resulting in inhomogeneity.

Extraction of Pesticides from the Sample

Extractions are performed using a solvent, or more frequently a system of solvents, by standard techniques. No universal extraction procedure of organochlorine compounds in all materials has yet been developed; each system requires its own extraction technique. The extraction stage is usually straightforward, but may require several steps.

Cleanup

It is essential to remove major interfering coextracted materials. The cleanup procedures fall into two general categories.

a) Partitioning: in which the pesticides are partitioned from one solvent into another for which they have a greater affinity than do the interfering substances.

b) Column (liquid-solid) chromatography: adsorption chromatography is frequently employed using florisil (a synthetic magnesium silicate compound), alumina, or carbon as the stationary phase. The degree of cleanup depends on adsorbent activity. A rigorous procedure must be followed for the preparation of the column, which, of course must be standardized.

Analysis

A gas chromatograph with an electron capture detector is used and is capable of producing good quantitative results for known pesticides. Qualitative identification is not so simple, due to the well-known nonspecificity of the electron capture detector, and is usually effected by either

a) chemical derivatization, followed by chromatography on the same column

or b) chromatography of the original compounds using a different column.

Careful use of procedures (a) and (b) will overcome problems of characterization caused by peak overlap which originates from entities with similar retention times.

Detection Limits and Precision

All of the organochlorine compounds of interest can be detected at a level of 0.002 ppm. The precision is dependent to a significant extent on the level and type of impurities: for a pesticide content below 0.005 ppm the precision obtained is usually of the order of ± 100%.

It is unavoidable that the organochlorine analysis is both time-consuming and beyond the scope of facilities in the average quality control laboratory.

Legal Aspects

Germany, Italy, Belgium, Holland, and the U.S.A. have independently introduced legislation controlling pesticide limits. Countries which have no definite legislation tend to refer to "Codex"[2] limits. There is no Codex limit for pesticide residues in molasses at present.

West Germany is considered to have imposed the strictest regulations. Any animal feedstuffs (such as molasses) exported to West Germany must be accompanied by a certificate for the Customs and Excise of West Germany, declaring that either

a) The legal limits of the organochlorine pesticides contents have not been exceeded (see table 1)

or b) the content(s) of the appropriate pesticide(s), if the legal limits have been exceeded.

TABLE 1--West German legislative limits on pesticides in animal feedstuffs

Compounds	Maximum pesticide content in Fats(mg/kg)	Grain(mg/kg)	Other Feedstuffs (mg/kg)
Aldrin and dieldrin	0.25	0.02	0.02
Chlordane	0.25	0.05	0.05
DDE, DDD, and DDT	0.6	0.03	0.05
Endrin	0.1	0.02	0.02
Heptachlor and heptachlor epoxide	0.25	0.02	0.03
BHC and lindane	2.5	0.1	0.1

Enforcement

Enforcement in West Germany is the responsibility of the police, with the backing of university or state facilities. It seems that imported feedstuffs are examined more frequently than nationally produced feedstuffs. German firms

[2] Joint FAO/WHO Food Standards Program. Codex Alimentarius Commission. 1969. Recommended international standard for white sugar, 17 pp.

are under no obligation to produce safety certificates for any authority, although one German firm may be sued by another under their "Unfair Trading" act.

In Italy also, the regulations are strict regarding organochlorine pesticides, their use on plants for human consumption being forbidden. It is accepted that foods may contain very small residues due to contamination from other sources. It is not clear if these regulations apply to animal feedstuffs; future legislation is likely to be more specific. However, in respect of contaminants the legal situation in Italy would benefit from clarification.

At present, the French are satisfied with Codex limits, but it is known that new legislation has been suggested.

In the U.S.A., special regulations are in force concerning the use of DDT. For example, meat animals may not be fed certain corn products which have been treated with DDT, unless the person so doing is in a position to determine the magnitude of DDT residues. Also, the application of DDT in any manner to the feed of dairy cows is absolutely forbidden. This infers that farmers are most likely to demand feedstuffs free from DDT. The effect on the molasses industry can easily be gaged.

The point at issue is whether the sugar industry should take the initiative by recommending realistic maximum levels before other countries legislate unreasonably against molasses.

HEAVY METAL CONTAMINANTS

This section is concerned with four metals, whose presence in large quantities in molasses would be undesirable. The metals investigated were copper, lead, zinc, and cadmium.

Legislation in England and Wales (foods for human consumption)

Copper

Small quantities of copper are essential for growth. No statutory controls are in force, but the recommended maximum limits are as follows:

Beverages (nonalcholic)	2.0 ppm
Most other foods	20 ppm

The nonalcoholic beverages are always associated with minimum contents of heavy metals.

Lead

Legislation passed in 1962 lays down the following limits:

Beverages (all)	0.2 ppm
Refined white sugar	0.5 ppm
Molasses and most other foods	5.0 ppm

Zinc

Small quantities are required for growth; the national daily intake for an adult has been estimated at 12 mg[3]. No legislation is in force, but the recommended maximum limits are as follows:

Beverages (nonalcoholic)	5 ppm
Most other foods	50 ppm

Cadmium

No legislation appears to exist for cadmium, but it is considered that 0.2 ppm is a reasonable maximum limit. There is an FAO/WHO provisional tolerable intake for man of 57-71 µg per day[4].

German Legislation

Recent legislation requires that the maximum concentration of lead in molasses and other animal feedstuffs be 0.1 ppm. Analytically this requirement is far less demanding than that for pesticide, but could be difficult to meet commercially, as it is not unknown for lead in molasses to exceed 5 ppm. The average value of lead in twenty-one samples analyzed in these laboratories was found to be 3.7 ppm. (see tables 4 and 5).

Legislation in the U.S.A.

There are no regulations in force regarding heavy metal content in the U.S.A. for molasses or any other animal feedstuff, although it is understood that the matter is under review at this time.

ANALYSIS

Classical

The ICUMSA methods described by Plews [5] are satisfactory for a small number of samples. The disadvantages are that there may be loss of sample during the ashing stage and the methods are laborious and time-consuming for a large number of samples.

Flame Atomic Absorption

For rapid analysis, the ashing stage may be excluded. The unknown sample is simply weighed, diluted with water, weighed again, then analyzed using the

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[3] O'Keefe, J. A. 1968. Bell and O'Keefe's sale of food and drugs (Great Britain). 14th Edition. 1584 pp. Butterworth and Co: Shaw and Sons, London.

[4] Joint FAO/WHO Expert Committee on Food Additives. 1972. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium. WHO Tech. Rept. Ser. No. 505, p. 32.

[5] Plews, R. W. 1970. Analytical methods used in sugar refining. 234 pp. Elsevier Co. Ltd, England.

method of additions. The spectroscopic conditions, dilution factors, and limits of detection are shown in table 2.

TABLE 2--Flame atomic absorption conditions

Metal examined	Wavelength (nm)	Slit width (μ)	Lamp current (mA)	Dilution factor	Detection limit (ppm)
Cadmium	228.8	200	3	8	0.2
Copper	324.7	100	3	5	0.15
Lead	217.0	300	6	5	0.4
Zinc	213.8	100	6	10	0.2

With regard to the dilution factor, clearly the detection limits for cadmium and zinc could have been improved if a dilution factor of five had been employed, but the detection limits shown in table 2 were adequate for the experiments at that time. A dilution factor of less than five is inadvisable because of blockage of the burner slot which leads to an unacceptable noise level in the flame, and corrosion of the burner in the long term.

Flameless Atomic Absorption

Use of this technique can lower the detection limit and increase the sensitivity. Theoretically, an ashing stage is not required, but matrix interference can cause suppression of the atomization signal. Results of some experiments conducted at the laboratories of Varian Associates[6] on a few samples of molasses showed that the atomization signal in lead determinations was indeed suppressed due to the presence of the molasses matrix; hence, to take full advantage of this sensitive technique, an ashing stage seems to be necessary in the determination of some heavy metals in molasses.

COMPARISON OF THE FLAME ATOMIC ABSORPTION AND ICUMSA METHODS

To demonstrate the precision and sensitivity of the flame atomic absorption methods, comparative analyses were carried out on one sample of molasses. Each determination was performed eight times and the results are shown in table 3.

[6] Varian Associates Instrument Division, 611 Hansen Way, Palo Alto, California 94303.

TABLE 3--Flame atomic absorption results

	Average value (ppm)	Standard deviation	Standard error of mean
Copper by flame atomic ab.[3]	8.4	0.3	0.1
Copper by ICUMSA	8.8	0.8	0.3
Zinc by flame atomic ab.[5]	9.0	1.0	0.3
Zinc by ICUMSA	8.4	1.3	0.5

It is seen that the flame atomic absorption method provides greater precision than the ICUMSA method, although the results are generally in good agreement. It should be noted that the flame atomic absorption method is by far the quicker, and we now recommend it for the routine analysis of molasses.

EXPERIMENTAL RESULTS

Twenty-one samples of molasses were analyzed using the flame atomic absorption technique and the method of additions. The results are shown in tables 4 and 5.

TABLE 4--Beet samples

Sample	Copper content (ppm)	Lead content (ppm)	Zinc content (ppm)
Dutch beet	10.8	0.6	48.6
Moroccan beet	2.2	1.6	17.5
Turkish beet	7.6	1.5	46.2
Bury beet	9.3	1.3	40.0

No cadmium was detected in any of the above samples i.e. cadmium content <0.2 ppm; the lower limit of detection for cadmium.

TABLE 5—Cane samples

Sample	Copper content (ppm)	Lead content (ppm)	Zinc content (ppm)
Guyana	6.5	0.7	11.2
South Africa	2.2	<0.4	2.7
Tunisia	7.6	9.8	17.0
Trinidad blackstrap	5.8	1.9	17.1
Puerto Rico	19.5	2.6	4.3
Sudan	10.4	1.1	11.6
Ecuador	15.0	2.3	8.8
Cuba	11.1	2.1	12.0
Java	14.0	2.6	13.0
Brazilian blackstrap	4.4	2.6	13.0
Dominican Republic	32.2	5.1	13.6
Jamaica	6.7	2.9	5.5
Pakistan	37.8	2.1	47.7
Mauritius	12.8	3.4	15.6
Egypt	9.7	3.5	12.1
Australian blackstrap	10.9	1.8	15.5
Iraq	3.3	2.2	5.4

No cadmium was detected in any of the above samples in table 5; hence, cadmium content <0.2 ppm.

From the above results it is seen that all but two of the samples, the Dominican and the Tunisian, would satisfy the British regulations with regards to lead content; also, due to lack of legislation, none of the samples would be barred in the U.S.A. In Germany, however, twenty of the twenty-one samples would not satisfy the regulations, and even the South African sample would need further analysis to determine whether the regulations would be satisfied.

The present state of legislation in this matter is clearly unsatisfactory and the situation would be vastly improved with the introduction of a greater uniformity in agreed maximum levels of heavy metals in molasses.

DISCUSSION

E. OBST (Amstar): Is gas-liquid chromatography the only method of analysis for pesticides, or is there anything simpler?

M. A. CLARKE (C.S.R.R.P.): Thin layer chromatography is frequently used for this sort of pesticide residue determination, but that technique is not nearly as accurate as GLC.

E. OBST: With GLC, do you have to use the electron capture detector, or can you use another type of detector?

M. A. CLARKE: To determine residues of phosphorus compounds, the electron capture detector must be used; flame ionization detectors will not do the job.

J. F. DOWLING (CPC International): In the heavy metals study done by the C.S.R.R.P. for lead, copper, and zinc[1], what kind of limits did you get on molasses?

M. A. CLARKE: We ran raw and refined sugars, but not molasses samples. Lead values ran from 0.1 ppm to 0.3 ppm in raw sugar, and an order of magnitude below that, from below 0.002 ppm to 0.03 ppm, in refined. One might expect the levels in molasses to be about ten times those in raw sugar, as the authors have found. In general, in foods, 2 ppm lead is a widely accepted limit.

The authors of this paper, Drs. Nicol and Morriss, feel that this agricultural chemical residue problem could blow up into a very expensive matter for many people, even if only as a question of proving that there is no problem, and they are very anxious to find out how people here think the problem should be handled. Does anyone have any experience or opinion on this?

W. W. BLANKENBACH (Chapman Associates): My only experience in this area occurred when the heptachlor residue problem showed up, about ten years ago. There was a rather hysterical reaction to this, all around the world. At that time, my company (B. C. Sugars) was producing dried beet pulp from beets that had been treated with heptachlor. Suddenly we got an order from the government forbidding the sale of this pulp, because it contained traces of heptachlor. Heptachlor use on beets was stopped, and the problem disappeared.

Up to that time, the analytical methods were not sensitive enough to show the presence of pesticides in significant amounts, and few people worried about residual quantities. With the development of gas chromatography, measurements at the part per billion level could be made for heptachlor and other chlorinated pesticides. Unfortunately, the GLC techniques were not well developed and quantitative results were frequently inaccurate, particularly when inexperienced chemists did the work. This was the case with the Canadian Government labs that had got caught up in the controversy over pesticide residues and had started to make determinations without adequate skills. Their results were both erratic and inaccurate, as we were able to prove when we employed a well-established research lab with many years of experience with GLC procedures to do our assays.

The point I would like to emphasize is the need for caution when reporting concentrations as minute as these. At the time I am speaking of, DDT had been sprayed around so much that almost everything was contaminated with chlorinated organic compounds and extreme precautions had to be taken to avoid erroneous results.

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[1] Clarke, M. A., Morris, N. M., Tripp, V. W., and Carpenter, F. G. 1974. Heavy metals in cane sugar products II. Proc. Sugar Ind. Technol. 33:91-101.

USE OF DIFFERENTIAL PULSE ANODIC STRIPPING FOR TRACE ELEMENTS IN SUGAR PRODUCTS

By P. Pommez and R. Cormier[1]

(Presented by R. Cormier)

ABSTRACT

Lead, copper, and cadmium are measured directly in raw sugar by using an electroanalytical technique known as differential pulse anodic stripping voltammetry. The three elements are detected simultaneously, and quantitation is accomplished by the method of standard addition that makes possible measurement of concentration in the ppb range.

INTRODUCTION

The determination of trace elements in sugar is the object of increasing attention. The time is coming when the quality control laboratory may have to run analyses for determination of elements at the ppm, ppb, or even sub-ppb levels. The question arises of the most appropriate technique to measure an element at the level predicted. This becomes rapidly a question of compromise between economics on one hand, and sensitivity, versatility and reliability on the other. Table 1[2] shows comparative virtues of the most routinely used methods for trace element analysis. All have advantages and limitations; however, the table clearly shows some of our reasons for choosing the anodic stripping voltammetry method. This method would be useful for the eventual study of interference between the matrix and the elements, that is, of the chelating effect of sugar on heavy metals. It has a high sensitivity in the parts and sub-parts per million range. The precision of the method is comparable to the precision of other existing methods. The cost of operation and of the original equipment are much lower than for any other methods.

The main limitation of anodic stripping voltammetry had been the number of elements detectable with the hanging mercury drop electrode traditionally used. However, this limitation is now offset by the development of new electrodes such as the glassy carbon electrode, with or without a mercury film formation on the electrode.

This paper will first give some general information on differential pulse anodic stripping voltammetry, then show some results obtained on a sample of raw sugar with the hanging mercury drop electrode, and finally, will show some very promising results obtained using a mercury film electrode.

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[1]Research and development manager and assistant research manager, Redpath Sugars, Ltd., P.O. Box 490, Montreal, Quebec, Canada H3C 2T5.

[2]Matson, W. R. 1974. Measuring and detecting trace metals. Food Eng. 46(8): 46-48.

TABLE 1--Comparative virtues of three methods

	Wet chemical colorimetric techniques	Anodic stripping voltammetry (ASV)	Atomic absorption spectroscopy
Sensitivity	ppm range	Parts and sub-parts per billion in 0.01g quantities	ppm or microgram level
Cost of operation per sample	$5 to $15	$3 to $6	$5 to $10
Original equipment cost	$5,000 to $10,000	$2,000 to $5,500 for basic equipment. Up to $10,000 with extenders and options	$7,000 to $25,000 depending on accessories

ANODIC STRIPPING VOLTAMMETRY (ASV)

Anodic stripping voltammetry is an electroanalytical technique which involves essentially three steps: preconcentration, equilibration, and stripping.

Preconcentration

A portion of the metal ions in solution is concentrated into a stationary mercury electrode. This is achieved by the application of a controlled potential which is selected to be more negative than the reduction potentials of all the various species to be determined. For the time this potential is applied, metal ions are reduced from their ionic state, in which they are present in solution, to the metallic form and then amalgamated with the mercury.

$$M^{n+} + ne^{-} \rightarrow M\text{-}Hg$$

However, if the ions are in a complexed state, some of the energy applied is used first to break this complex:

$$\left[M\text{-}L_x\right]^{n+} \rightarrow M^{n+} + x\,L.$$

Equilibration

This permits a uniform distribution of the amalgam to be obtained.

Stripping

At the end of the equilibration stage a linearly increasing potential gradient is applied. As the potential is made more positive the various metals present in the amalgam are oxidized out, each beginning the oxidation process at its own redox potential and redissolving in the solution as ions.

$$M\text{-}Hg \rightarrow M^{n+} + n\,e^-$$

Current is generated by this reaction; the amount of current depends on the concentration of each of the materials being oxidized, thus giving a typical waveform polarogram.

This basic stripping technique has been known for many years. Its use has, however, been considerably limited, because the technique suffers a number of severe practical limitations arising from the problems encountered when very long deposition times are employed to increase sensitivity. The main problem arises from a high capacitance current, which results from the charging of the electrode-solution interface, as compared to the Faradaic current, which is the signal to be detected. One solution to that interfering current is to increase the signal-to-background ratio by a technique which discriminates against the capacitance current. This is the method used in differential pulse anodic stripping.

DIFFERENTIAL PULSE ANODIC STRIPPING (DPAS)

The DPAS technique makes use of the concentration step of ASV. However, at the stripping stage, a small amplitude pulse is superimposed upon the linear gradient used in ASV, as shown on figure 1[3].

The pulse duration is 56.7 msec, the first 40 msec of which are used to allow the charging current to decay to a negligible value. The last 16.7 msec are used to measure the Faradaic current. The current flow is sampled just before application of the pulse and again during the last portion of the pulse. The difference between the two current samples is displayed on the recorder, and the normal dc stripping waveform becomes a peak-shaped read-out that is more convenient for quantitation.

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[3]Applied Research Corporation. 1973. Modern analytical polarography workshop manual. Section 3, p. 7. Princeton, N. J.

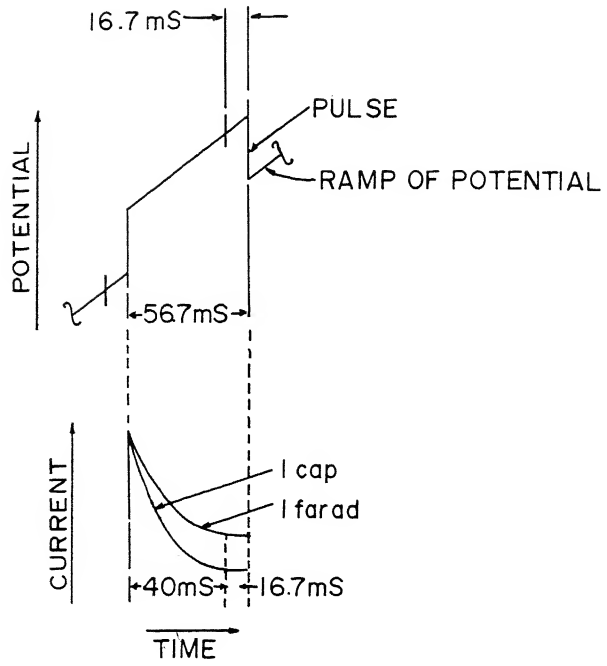

FIGURE 1--Differential pulse[2].

ELECTRODES

Three types of electrodes are available:

1. The solid electrodes made with platinum, gold, silver, or carbon.
2. The hanging mercury drop electrode (HMDE), which is essentially a drop of mercury of constant size, held for the duration of the test.
3. The mercury film electrode (MFE). A mercury film is formed on the surface of a solid electrode which can be either a wax impregnated graphite electrode or a glassy carbon electrode[4,5].

Most of the results reported herein were obtained with the HMDE. Some preliminary results obtained with the MFE on a glassy carbon electrode are also included.

INSTRUMENTATION

Apparatus

The polarograph used was a Princeton Applied Research instrument, model 174 in conjunction with a Brinkman recorder, model 2571.

[4]Florence, T. M. 1970. Anodic stripping voltammetry with a glassy carbon electrode mercury-plated in situ. J. Electroanal. Chem. Interfacial Electrochem. 27: 273-281.

[5]Copeland, T. R., Christie, J. H., Osteryoung, R. A., and Skogerbea, R. K. 1973. Analytical applications of pulsed voltammetric stripping at thin film mercury electrodes. Anal. Chem. 45: 2171-2174.

Other components required:

1. A deoxygenating device. Oxygen dissolved in the solution may interfere with the process and must be removed. This is achieved by bubbling oxygen-free nitrogen through the solution for approximately 3 minutes. The nitrogen is first passed through a solution with zinc amalgam, then through a column filled with silica gel and finally through the electrolyte used in the cell in order to saturate the nitrogen with the electrolyte.
2. A polarographic cell, PAR model 9300-9301.
3. A synchronous stirrer with an on-off switch.
4. A reference electrode: saturated calomel reference electrode PAR model 9311.
5. A reference electrode salt bridge tube, PAR model 9332, to provide isolation of the reference electrode from the test solution. The bridge incorporates a low leakage fitted glass junction.
6. A platinum wire counter electrode, PAR model 9312.
7. Outgassing tube, PAR model 9331. A two-way teflon stop-cock directs nitrogen through sample solution for outgassing, or over solution for gas blanket.
8. The hanging mercury drop assembly, containing a mercury micro-feeder (Metrohm Model EM5-03). The mercury micro-feeder consists of a capillary filled with mercury, and a piston of which the stroke is controlled by a micrometer. A drop of mercury is extruded from the capillary; the size of the drop depends on the number of divisions the micrometer has been turned. Our work has been conducted with 4 micrometer divisions which corresponds to a drop surface area of $2.22 \pm 0.07 mm^2$. An alternate to the HMDE is a glassy carbon electrode, PAR model 9333. It is formed of very hard vitreous carbon, polished to a mirror-like finish. The surface area is approximately $28 mm^2$. This electrode was employed for part of the current study.
9. Reagents: It is essential that all reagents be ultra pure. Reagents used were BDH, aristar grade. Triple distilled mercury was used.

ANALYTICAL PROCEDURE

Sample preparation

It was decided to forego prior digestion of the sample, since wet ashing and incineration are both well known sources of errors. Instead, a liter of a 5% solution of the raw sugar sample in the chosen electrolyte was prepared. By using a large quantity of sugar (50 g), better sample representation is achieved. On the other hand, it is difficult to work at a higher sucrose concentration (above 5%) as the increase in viscosity affects the diffusion of ions, and consequently the sensitivity of the method[6].

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[6]Strocchi, P. M., and Gliozzi, E. 1951. The metal complexes of sucrose. I. Polarographic and conductometric studies. Ann. Chim. (Rome), 41: 689-696.

Two electrolytes were used to prepare the sugar solution: hydrochloric acid, 1.0M, and ammonium hydroxide-ammonium citrate, 0.1M. By measuring the elements present in sugar in two different electrolytes, it was hoped to evaluate the sequestering effect of sucrose on those elements.

Deposition

Twenty ml of the sugar solution are pipetted into the polarographic cell. Oxygen free nitrogen is then bubbled for 10 minutes. At the end of the outgassing period, the valve is positioned to permit a nitrogen blanket over the solution, to prevent reabsorption of atmospheric oxygen by the solution.

A potential equal to -0.750V (versus the standard calomel electrode) is applied to the working electrode, and at the same time a laboratory timer is started. At the end of the selected time, stirring is stopped and the solution allowed to stand for 30 seconds. At the end of the rest period the stripping procedure is initiated and recorder started.

The conditions used for the stripping were as follows:

Deposition time --	3 to 10 minutes depending on the element concentration.
Scan rate --	1 to 2 mV/second.
Pulse frequency --	1/second.
Modulation amplitude --	25 to 50 mV.
Current range --	1 to 5 μA.

At the end of the stripping, the potential is returned to the initial potential. A new drop is extruded and a fixed volume of a standard solution of the metal of interest at a known concentration is added. The entire identical procedure is repeated and the stripping polarogram recorded.

Quantitation

The concentration of the element in the sugar solution is derived from the height of the peaks before and after standard addition, using the relation:

$$C_x = \frac{(C_s v h)}{HV + (H-h)V},$$

where C_x = concentration of the element in the 5% sugar solution expressed in ppm,

V = volume of 5% sugar solution in the cell,

C_s = concentration of standard in ppm,

v = volume of standard (mls),

h = height of peak before addition (mm),

and H = height of peak after addition (mm).

RESULTS

Hanging Mercury Drop Electrode

Lead and Copper in Electrolyte

The supporting electrolytes used to prepare the sugar solution are always somewhat contaminated by heavy metals; consequently, lead and copper had to be determined. If an element is in sugar at a concentration of 0.20 ppm on solids, it has a concentration of 0.01 ppm in a 5% sugar solution. The effect of the electrolyte contamination is then not negligible, even if the element is at the ppb level in the electrolyte. Figure 2 shows the polarograms corresponding to the determination of lead and copper in 1 N HCl. Table 2 gives the results of the lead and copper determination in both citrate and hydrochloric acid.

TABLE 2--Lead and copper in supporting electrolytes (ppb)

	Cu	Pb
HCl, 1 N	3.5	1.0
NH_4OH 0.1 M $(NH_4)_3C_6H_5O_7$ 0.1 M	2.3	1.6

Lead and Copper in Raw Sugar

Two different electrolytes were used in order to evaluate the possible sequestration of some of the metal atoms by the sucrose molecule. Sucrose is known to exert a chelating effect, probably through the available unshared pairs of electrons of the oxygen atoms. When sucrose is in a basic environment, such as the citrate electrolyte, the oxygen atoms are free to coordinate their electron doublet with positive ions[7]. However, in an acidic medium, the hydroxyl group is protonated and the metallic ions remain uncomplexed, as shown in figure 3.

[7]Copeland and others, cited in footnote 5.

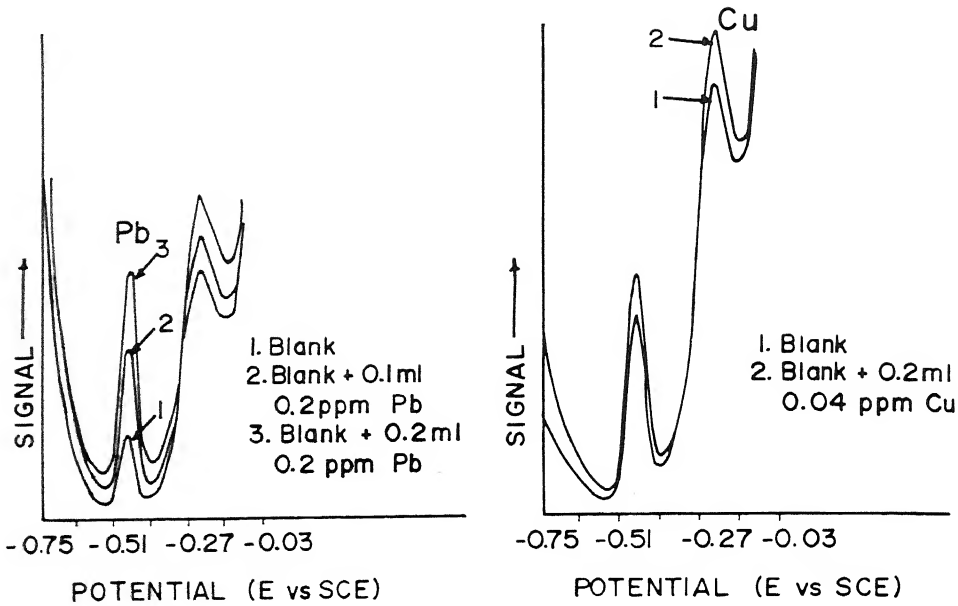

FIGURE 2--Pb and Cu in blank.

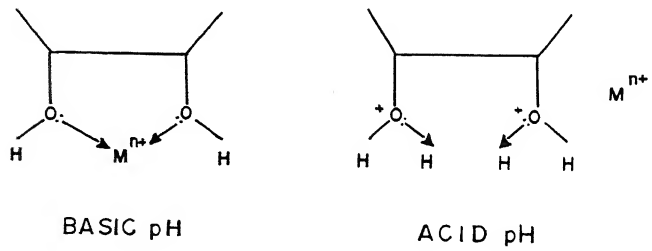

FIGURE 3--Chelating effect.

Figure 4 shows the polarogram obtained for copper and figure 5 that for lead in sugar in hydrochloric acid electrolyte. The tailing effect is particularly notable.

Figure 6 shows the corresponding polarogram in the citrate electrolyte, and figure 7 the determination of cadmium in this electrolyte.

There is still some tailing in the citrate electrolyte; however, the resolution is much better and the cadmium peak appears well defined in this buffer although it was not even visible in the acidic medium.

Consideration of the data in table 3 reveals that the range of use for citrate electrolyte is somewhat limited. The table gives metal concentrations in a raw sugar in citrate and in HCl by DPAS, and in the same raw sugar by atomic absorption spectroscopy.

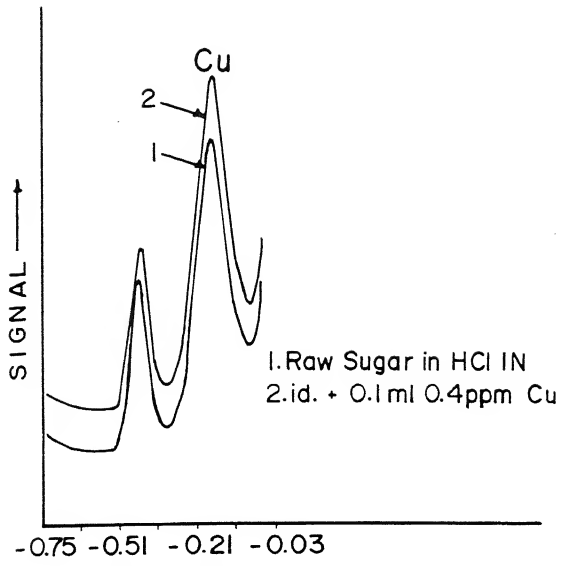

FIGURE 4--Cu in raw sugar.

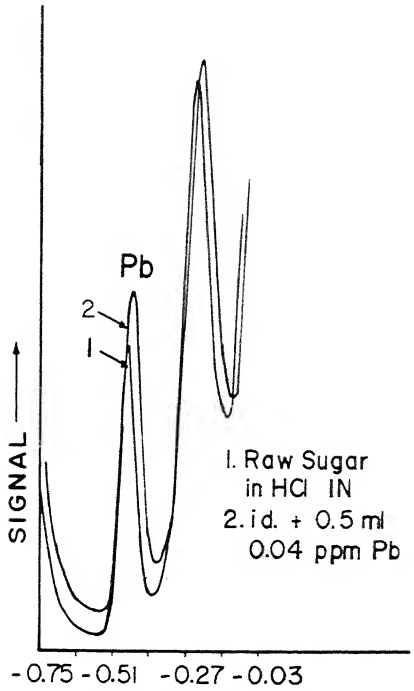

FIGURE 5--Pb in raw sugar.

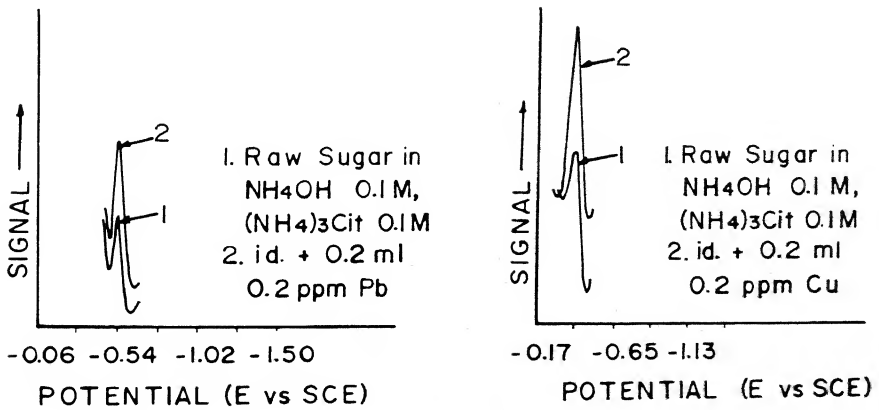

FIGURE 6--Pb and Cu in raw sugar.

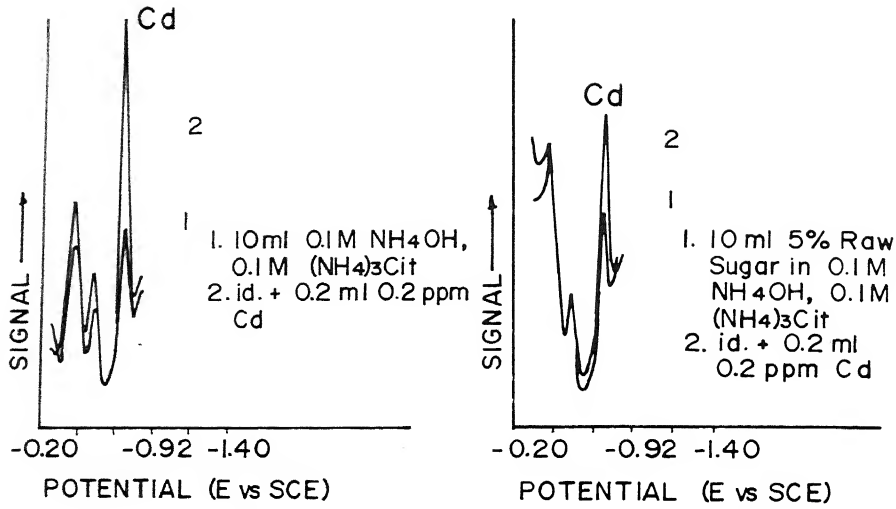

FIGURE 7--Cd in raw sugar.

TABLE 3--Cu and Pb in raw sugar (ppb)

	DPAS		Atomic absorption
	Citrate	HCl	
Cu	242	516	440[1] 420[2]
Pb	132	135	170
Cd	57	-	13

[1]By method of additions
[2]By standard curve

The cadmium result, by DPAS is only an approximation as the test was not repeated. The large difference observed in copper concentration in citrate and hydrochloric acid indicates the complexation of copper with citrate, while this phenomenon is not observed with lead. The difference between DPAS values in HCl and atomic absorption values might be due to the more representative nature of the sample solution used in DPAS. These analyses are for nanogram quantities of these elements, and the presence of tiny molasses lumps in a small sample, or even a different particle size distribution of the sample used for the determination, may drastically affect the final figures.

Mercury Film Electrode (MFE)

These results are only preliminary as work with the glassy carbon electrode is still in progress.

The basic technique is the same as that for the HMDE except that the mercury film is made in situ instead of in a drop of mercury. One ml of a solution of 100 ppm mercuric ions is introduced into the sample solution. The deposition potential is applied as before, but in this case the mercury is reduced simultaneously with the metal ions, and an amalgam film of about 1μ to 100μ is formed on the glassy carbon surface. The surface area is about 28mm^2 as compared to about 2.5mm^2 on the mercury drop. In addition to the increased surface area, internal diffusion within the drop is now eliminated; these improvements result in much better resolution and sensitivity, as shown on the polarograms for lead in blank (figure 8) and in raw sugar (figure 9). The sugar solution is only 0.5% concentration, instead of 5% for HMDE, and the current range 10μA instead of 2μA for HMDE. Further improvement may be achieved by using a rotating electrode which gives a more uniform layer of mercury.

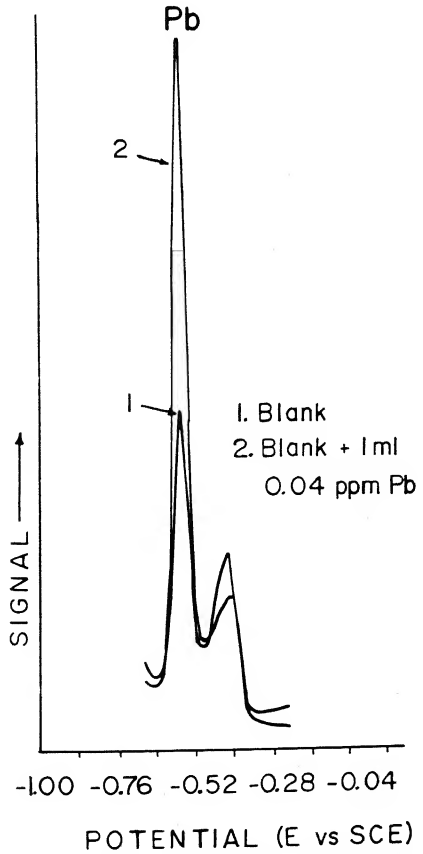

FIGURE 8--Analysis of lead with glassy carbon electrode.

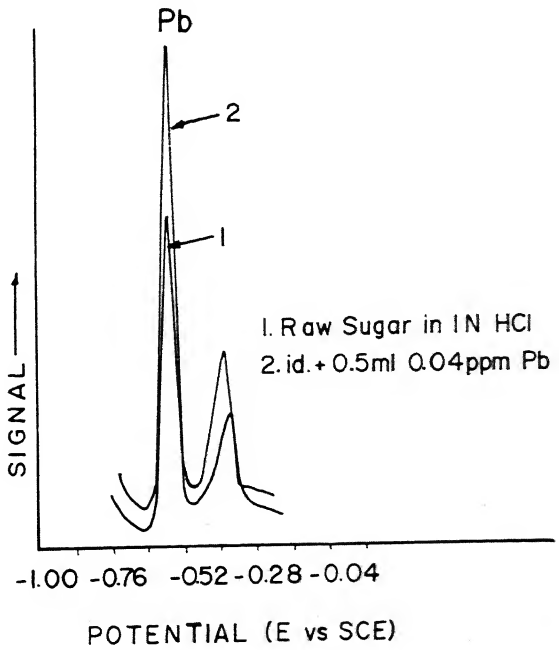

FIGURE 9—Analysis of lead with glassy carbon electrode.

Its manufacturers claim that the MFE permits extension of the voltage range covered i.e. the number of elements which can be detected. Our tests have not shown this effect; however, it is possible that the use of another electrolyte may give better results.

CONCLUSION

Differential pulse anodic stripping is an extremely attractive method for analysis of sugars. Lead, copper, cadmium and possibly zinc can be detected at the ppb level with a hanging mercury drop electrode. The mercury film electrode seems very promising and should make possible determination of more elements at ppb and sub-ppb levels.

ACKNOWLEDGEMENT

The authors wish to thank the Cane Sugar Refining Research Project which carried out the atomic absorption determination. Our thanks are also due to J. Dilaj for his major contribution to the analytical part of the work. We would also like to thank Redpath Sugars Ltd. for the authorization to publish this paper.

DISCUSSION

E. O'NEILL (Amstar): Would this technique be useful for running routine trace analyses in a control laboratory?

R. CORMIER: There are several criteria to consider. The cost of the equipment, and of a single analysis, compares very well to the cost of other methods available. The method does not require a particularly skilled operator --a technician can readily learn the procedure. Once the equipment is set up, the only change for each determination is to make a fresh drop of mercury. The time required per sample is very short; the method is reproducible, and multiple readings are not required. The installation of the equipment is quite simple--it can all be installed in a morning. I think that this technique would be a very fine method for use in a routine control laboratory.

M. A. CLARKE (C.S.R.R.P.): I agree with Mr. Cormier that this would be a good method for a quality control lab. As he says, the initial investment, and continuing costs, in equipment are low; the method is rapid, reproducible, and easily performed. It is especially recommended for a laboratory that does not already possess equipment for metal analysis.

R. CORMIER: An additional point, which might make this method more attractive to sugar laboratories, is that we are working out a method for iron determination. This is very important in sugar work, and is not usually feasible by polarographic techniques, since iron does not form an amalgam with mercury. It may, however, be determined using another electrolyte. We chose the electrolyte that we did, HCl, because it was one of the purest chemicals on the market. Phosphoric acid, for example, has traces of almost all metals in it, although it is easily purified by electrolysis. This polarograph can also perform electrolysis--it's simply a matter of changing the connections.

Because of this, you don't need other equipment to purify an electrolyte; we have been making a survey of the use of various electrolytes, and the preliminary screening results are very promising.

CARBOHYDRATE CHANGES IN INVERT SIRUPS

By Violeta S. Velasco and Joseph F. Dowling[1]

(Presented by Violeta S. Velasco)

ABSTRACT

It has been shown that medium and total invert sirups do not always contain equal quantities of dextrose and levulose resulting from the inversion of sucrose. Both gas-liquid and paper chromatographic analyses revealed higher concentrations of dextrose than levulose.

Investigation of the inversion reaction at low pH and high temperature showed that levulose is transformed into possible difructose dianhydrides.

INTRODUCTION

It has generally been assumed that both medium and total invert sirups contain equal quantities of dextrose and levulose resulting from the inversion of sucrose; however, a recent survey of these commercially available sirups revealed that dextrose is present in greater quantity than levulose.

CHROMATOGRAPHIC ANALYSIS

Data obtained by paper chromatographic analyses[2] for various invert sirups produced from both beet and cane sugar by acid or ion-exchange inversion are shown in table 1. Further investigations revealed that so-called "higher sugars" (multiple sugar units) were present in these sirups. Their paper chromatographic flow rates were slower than that of sucrose and corresponded to those of maltooligosaccharides found in corn sirups.

Ten samples of various production batches of total invert sirup produced at our Yonkers Refinery were analyzed by the paper chromatographic method at Moffett Technical Center-Argo[3], and by a gas-liquid chromatography method[4] at Yonkers. A comparison of the data obtained by the two methods, when the total sugar content is taken as only dextrose, levulose, and sucrose, indicated fairly good agreement between the two laboratories, as shown in table 2.

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[1]Chemist and technical director, Industrial Division, CPC International Inc., Federal St., Box 509, Yonkers, N. Y. 10702.

[2]Corn Industries Research Foundation, Inc. Standard Analytical Methods of Member Companies. 1957. Method E-62.

[3]Moffett Technical Center, CPC International, Inc., Argo, Illinois 60501.

[4]Velasco, V. S., Heisler, M., and Dowling, J. F. 1971. Quantitative measurements of sugar by gas-liquid chromatography. Proc. Tech. Sess. Cane Sugar Refin. Res. 1970: 61-81.

TABLE 1--Sucrose, dextrose, and levulose in medium and total invert sirups by paper chromatography

Samples	% Sucrose	% Dextrose	% Levulose	Difference % Dextrose-% Levulose
Medium invert				
1	50.6	26.1	23.3	2.8
2	50.9	25.4	23.4	2.0
3	45.2	27.5	26.9	0.6
4	47.2	26.8	26.0	0.8
5	47.5	27.3	25.9	1.4
6	48.5	25.7	25.3	0.4
Total invert				
1	5.8	48.6	45.6	3.0
2	9.4	45.4	42.5	2.9
3	3.9	48.5	43.7	4.8

TABLE 2--Sucrose, dextrose and levulose in total invert samples. Paper chromatography (PC) vs. gas-liquid chromatography (GLC)

	% Levulose			% Dextrose			% Sucrose		
Samples	PC	GLC	PC-GLC	PC	GLC	PC-GLC	PC	GLC	PC-GLC
1	47.4	48.8	- 1.4	50.5	49.7	0.8	2.0	1.4	0.6
2	46.6	47.3	- 0.7	48.3	47.6	0.7	5.1	5.1	0.0
3	47.8	48.4	- 0.6	49.8	49.5	0.3	2.4	2.1	0.3
4	48.4	47.9	0.5	50.0	50.7	- 0.7	1.5	1.3	0.2
5	47.0	46.6	0.4	49.7	49.7	0.0	3.3	3.7	- 0.4
6	48.3	47.5	0.8	49.7	49.9	- 0.2	2.0	2.6	- 0.6
7	48.4	48.5	- 0.1	50.0	50.2	- 0.2	1.5	1.4	0.1
8	45.2	44.4	0.8	46.8	48.0	- 1.2	8.1	7.6	0.5
9	47.9	47.5	0.4	50.8	51.6	- 0.8	1.2	0.9	0.3
10	48.3	48.0	0.3	50.3	51.3	- 1.0	1.4	0.7	0.7

For all the samples tested, the dextrose content was significantly higher than that of levulose (table 3). Thus it is believed that during the inversion of sucrose, under present sugar refining conditions, levulose is at the same time undergoing other chemical reactions.

TABLE 3--Difference in dextrose and levulose concentration for total invert

Total invert samples	Dextrose minus levulose paper chromatography	Dextrose minus levulose gas-liquid chromatography
1	3.1	0.9
2	1.7	0.4
3	2.0	1.1
4	1.6	2.8
5	2.7	3.1
6	1.4	2.4
7	1.6	1.7
8	1.6	3.6
9	2.9	4.1
10	2.0	3.3
	+ 2.06	+ 2.34

INVERSION RATE CHANGE WITH pH AND TEMPERATURE

Samples of liquid sucrose were adjusted in the laboratory to various pH's with hydrochloric acid, and inversion carried out at 110° and 130°F. The dextrose and levulose content was determined by the GLC method, and even at 110°F the dextrose content was higher than that of the levulose (table 4). Solutions of 50% sucrose adjusted to various pH's were stored at room temperature, and no major destruction was noted (table 5). It appears that the destruction of levulose is very dependent upon both pH and temperature.

TABLE 4—Dextrose and levulose content of sucrose solutions of acid pH at high temperatures

Temperature °F.	pH	Time of inversion (h)	% Dextrose	% Levulose	Difference dextrose-levulose
110°	1.4	24	30.2	28.3	1.9
130°	1.4	2	20.2	18.6	1.6
130°	1.4	3	25.0	22.9	2.1
130°	1.4	4	27.7	26.6	1.1
130°	1.7	4	19.0	17.8	1.2

TABLE 5--Dextrose and levulose content of 50% sucrose solutions stored at acid pH at room temperature

pH Samples	Days in test	% of Total sugars		% in Sample	
		Levulose	Dextrose	Levulose	Dextrose
1.5	4	20.5	21.2	10.4	10.8
	12	43.5	42.8	21.2	20.8
2.0	4	4.7	5.2	2.2	2.4
	12	15.0	15.1	5.5	5.5
2.5	4	1.5	1.7	0.7	0.8
	12	5.8	6.1	2.4	2.6

To investigate further this difference in dextrose and levulose, a 50% solids solution was prepared by dissolving equal weights of the two sugars in water, mixing, and then dividing the solution into three samples. One sample served as a control and was at pH 4.7. The other two samples were adjusted to pH 2.4 and pH 1.0 with hydrochloric acid. All three were then placed in an oven at 90°F. There appeared to be no major destruction after four days at this temperature (table 6). The samples were then transferred to a 130°F oven for the remainder of the test.

TABLE 6--Dextrose and levulose content of dextrose-levulose solutions at various pH's and temperatures, as percent of dextrose & levulose

Days in test	Temperature °F	4.7 pH Sample		2.4 pH Sample		1.0 pH Sample	
		% Levulose	% Dextrose	% Levulose	% Dextrose	% Levulose	% Dextrose
0	-	49.9	50.1	50.6	49.4	49.9	50.1
4	90°	-	-	49.9	50.1	49.7	50.2
6	130°	49.1	50.9	48.6	51.4	47.9	52.1
13	130°	50.4	49.6	49.5	50.5	46.1	53.9
18	130°	-	-	51.0	49.0	44.2	55.8
19	130°	50.0	50.0	51.4	48.6	-	-

Control Sample

During the 19 days of testing, the pH dropped from 4.7 to 3.0, and there was a slight color buildup (0.7 Horne color at 19 days). The dextrose-levulose concentrations varied during the test, but an overall evaluation indicated no

major changes in levulose. Another means of evaluating the data is to examine the percent sugars in solution. This is found by dividing the weight of sugar found by the GLC method by the total weight of sample derivatized (table 7). If the data obtained for this sample are averaged, almost equal amounts of dextrose and levulose are obtained--indicating little to no changes in the two sugars.

TABLE 7--Dextrose and levulose content of dextrose-levulose solutions at various pH's and temperatures, as percent of total sample

Days in test	Temperature °F	4.7 pH Sample		2.4 pH Sample		1.0 pH Sample	
		% Levulose	% Dextrose	% Levulose	% Dextrose	% Levulose	% Dextrose
0	-	26.8	26.9	26.6	26.0	26.5	26.6
4	90	-	-	26.6	26.8	25.4	25.7
6	130	26.1	27.1	25.0	26.4	24.0	26.0
13	130	26.1	25.7	25.6	26.2	21.5	25.2
18	130	-	-	27.8	26.8	19.1	24.1
19	130	26.1	26.0	27.7	26.2	-	-
Average		26.3	26.4	26.6	26.4	23.3	25.5

Data reported as percent sugar in solution.

2.4 pH Sample

During heating, the pH remained at 2.4, and there was a slight color buildup (0.7 Horne color at 19 days). Initially it appeared that levulose was being destroyed, but on days 18 and 19, the levulose concentration was greater than that of dextrose (table 7). This reversal could be due to analytical error, even though the results were reproducible. It could also be caused by the formation of a compound with retention time similar to levulose, or by the formation of one of the levulose isomers not generally measured. Dextrose remained fairly constant during the entire test, while levulose was varying. These samples are still under test, and more analyses are certainly required to enable us to explain better what is taking place in this particular system.

1.0 pH Sample

This sample remained at 1.0 pH during heating, and exhibited a large development of color (14.0 Horne color at 19 days). There was a steady decline in levulose concentration during the entire test (table 7), and dextrose also started to decline after 13 days. This sample started to exhibit GLC peaks with retention time similar to sucrose when tested on the 13th day. Thus, it is quite evident that some destruction is taking place in this sample, and that levulose concentration is changing at a greater rate than dextrose.

The GLC method included conversion of the sugar into its silyl ether derivative using pyridine as a solvent. This solvent, however, caused multiple products because of anomerization of sugars. This has posed some problems, especially in the quantitation of dextrose [5]. A minor anomer of levulose which overlapped with the α-dextrose peak necessitated corrections. Any change in concentration of this anomer might result in variations in GLC data. To eliminate this problem, the sugars were first treated with hydroxylamine hydrochloride, and the resulting oximes were converted to trimethylsilyl ethers (see Appendix). Multiple peaks due to tautomeric forms of reducing sugars were eliminated, and single peaks were obtained.

TABLE 8--Dextrose and levulose content as measured by the oxime-TMS method of dextrose-levulose solutions and sucrose solutions stored at 130° F and at various pH's

	Dextrose-levulose solution						Sucrose solution	
	pH 2.0		pH 1.5		pH 1.0		pH 1.0	
Days in test	% Lev.	% Dex.	% Lev.	% Dex.	% Lev.	% Dex.	% Lev.	% Dex.
0[1]	30.2	29.4	30.2	29.4	30.2	29.4		
0[2]	30.1	29.3	30.5	30.0	30.5	29.9		
3	28.7	29.1	28.2	29.0	26.0	28.6	26.9	30.0
4	28.1	28.3	27.5	28.4	24.0	27.0	24.8	28.4
10	27.9	28.9	26.2	28.5	20.9	27.2	20.4	27.8
Average	29.0	29.0	28.5	29.0	26.3	28.4	24.1	28.8
S.D.[3]	0.27	0.19	0.35	0.28	0.50	0.43	0.43	0.47

[1]Levulose and dextrose at start.
[2]Levulose and dextrose after pH adjustment.
[3]S.D. (standard deviation) = $\sqrt{(1/2N)(\text{sum of differences})^2}$

The experiments were repeated using this technique, and the results are shown in table 8. The dextrose-levulose solutions were adjusted to pH 2.0, pH 1.5, and pH 1.0 while the sucrose solution was kept at pH 1.0, and then stored at 130°F. These results indicated the same trend as those obtained by straight silylation of sugars. Slight levulose destruction was also occurring at pH 2.0 and, to a greater extent, at pH 1.0, as shown in figure 1. Precision of the oxime-TMS method was measured by determining standard deviation[6]. Each of the percent sugars figures given in table 8 was an average of duplicate analyses. By taking differences between each pair, standard deviations were calculated for each set; results showed a range from 0.19% to 0.50%.

[5]Velasco, Heisler, and Dowling, cited in footnote 4.

[6]Youden, J. W. 1951. Statistical methods for chemists. p. 16. Wiley Co., N. Y.

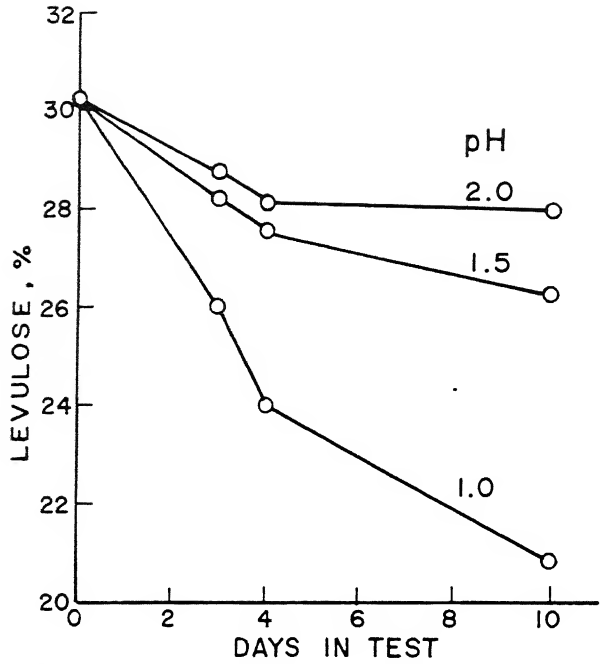


FIGURE 1--Levulose in dextrose-levulose solution stored at 130°F at various pH's.

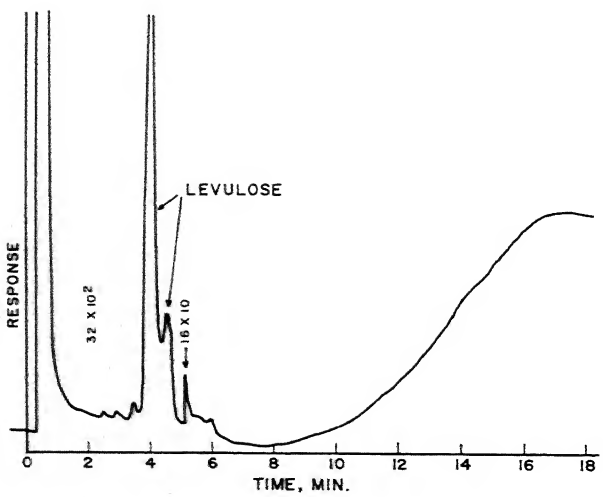


FIGURE 2--Levulose solution, pH 4.2, no heat.

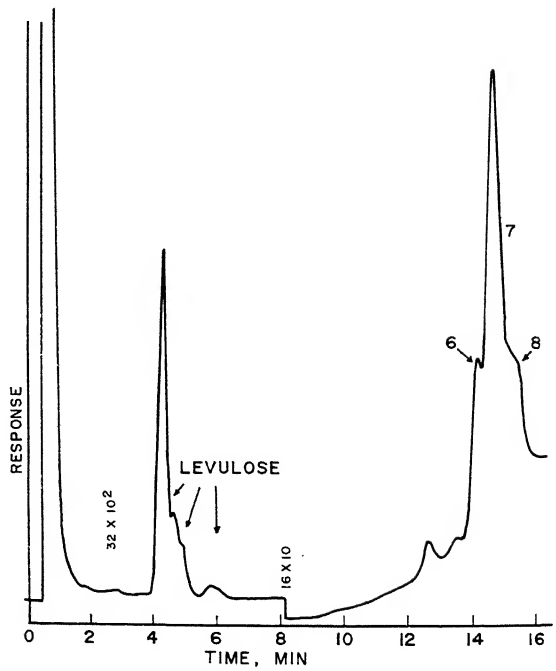


FIGURE 3—Levulose solution, pH 1.2, heated 5 h at 180°F.

The GLC method included conversion of the sugar into its silyl ether derivative using pyridine as a solvent. This solvent, however, caused multiple products because of anomerization of sugars. This has posed some problems, especially in the quantitation of dextrose [5]. A minor anomer of levulose which overlapped with the α-dextrose peak necessitated corrections. Any change in concentration of this anomer might result in variations in GLC data. To eliminate this problem, the sugars were first treated with hydroxylamine hydrochloride, and the resulting oximes were converted to trimethylsilyl ethers (see Appendix). Multiple peaks due to tautomeric forms of reducing sugars were eliminated, and single peaks were obtained.

TABLE 8--Dextrose and levulose content as measured by the oxime-TMS method of dextrose-levulose solutions and sucrose solutions stored at 130° F and at various pH's

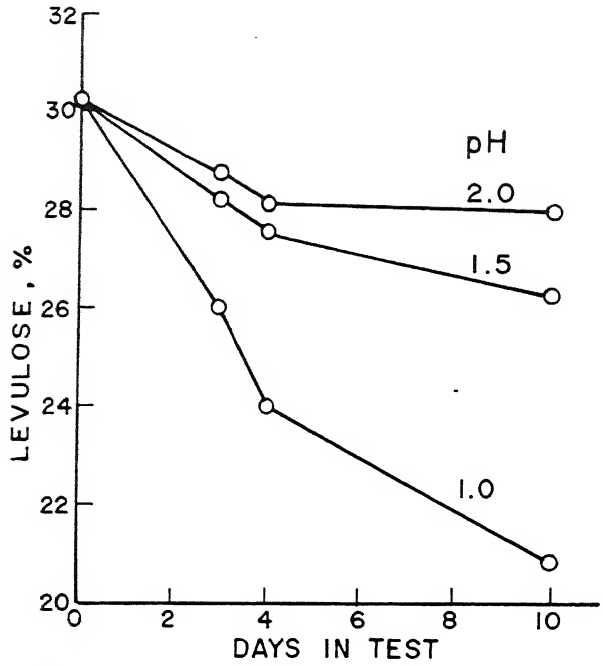
	Dextrose-levulose solution						Sucrose solution	
	pH 2.0		pH 1.5		pH 1.0		pH 1.0	
Days in test	% Lev.	% Dex.	% Lev.	% Dex.	% Lev.	% Dex.	% Lev.	% Dex.
0[1]	30.2	29.4	30.2	29.4	30.2	29.4		
0[2]	30.1	29.3	30.5	30.0	30.5	29.9		
3	28.7	29.1	28.2	29.0	26.0	28.6	26.9	30.0
4	28.1	28.3	27.5	28.4	24.0	27.0	24.8	28.4
10	27.9	28.9	26.2	28.5	20.9	27.2	20.4	27.8
Average	29.0	29.0	28.5	29.0	26.3	28.4	24.1	28.8
S.D.[3]	0.27	0.19	0.35	0.28	0.50	0.43	0.43	0.47

[1]Levulose and dextrose at start.
[2]Levulose and dextrose after pH adjustment.
[3]S.D. (standard deviation) = $\sqrt{(1/2N)(\text{sum of differences})^2}$

The experiments were repeated using this technique, and the results are shown in table 8. The dextrose-levulose solutions were adjusted to pH 2.0, pH 1.5, and pH 1.0 while the sucrose solution was kept at pH 1.0, and then stored at 130°F. These results indicated the same trend as those obtained by straight silylation of sugars. Slight levulose destruction was also occurring at pH 2.0 and, to a greater extent, at pH 1.0, as shown in figure 1. Precision of the oxime-TMS method was measured by determining standard deviation[6]. Each of the percent sugars figures given in table 8 was an average of duplicate analyses. By taking differences between each pair, standard deviations were calculated for each set; results showed a range from 0.19% to 0.50%.

[5]Velasco, Heisler, and Dowling, cited in footnote 4.

[6]Youden, J. W. 1951. Statistical methods for chemists. p. 16. Wiley Co., N. Y.



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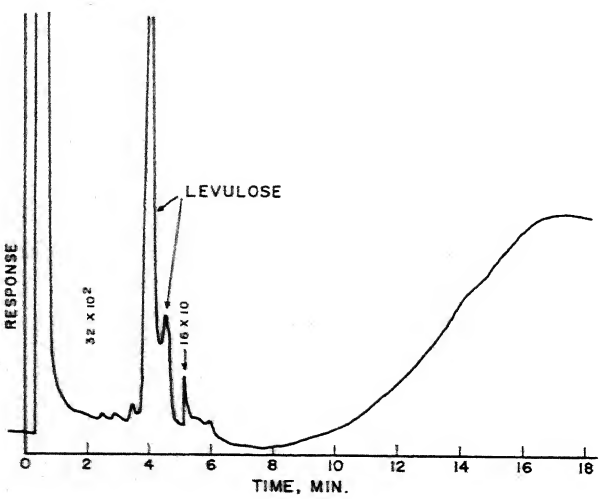

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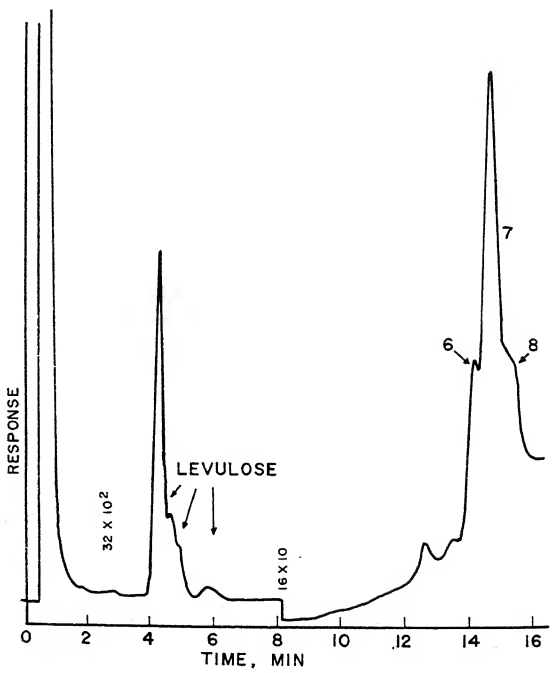

FIGURE 3--Levulose solution, pH 1.2, heated 5 h at 180°F.

At the present time, two methods for inverting sucrose--namely, acid inversion or invertase--are used in standard methods of analyses for sucrose. In standardizing Fehlings solution, it is recommended that a standard invert solution be prepared by inverting a known quantity of sucrose with hydrochloric acid (at approximately 0.6 pH) [7]. A solution of invert (9.5g sucrose, 100 ml water, and 5 ml of concentrated hydrochloric acid) was prepared by this method and stored at room temperature. As can be seen in table 9, levulose is once again declining in concentration with time. We have prepared other similar solutions and have had difficulty in obtaining a 50/50 mixture of levulose and dextrose.

TABLE 9--Dextrose-levulose content of standard invert solution at room temperature, prepared by acid method

	% Of total sugar		% In solution	
Days stored at room temperature	% Levulose	% Dextrose	% Levulose	% Dextrose
1.5	47.8	52.2	4.6	5.1
2.5	49.3	50.7	4.9	5.0
9	46.0	54.0	4.2	4.9
10	49.1	50.9	4.8	5.0
14	45.6	54.4	4.1	4.9
16	45.3	54.7	4.2	5.0
Average	-	-	4.5	5.0

However, solutions inverted with invertase more resembled a 50:50 mixture of the two sugars as expected (table 10). Based on this limited testing, it would appear that invertase is the better analytical tool for inverting sucrose, and that in preparing standard invert solutions, it would be preferable to use a pure dextrose and levulose mixture, or to use invertase as the inverting agent.

Formation of Unknown Sugars

In order to investigate further the possibility of higher sugars forming in the pH 1.0 sample of levulose and dextrose heated at 130°F, a 50% solution of levulose was prepared, and half the sample adjusted to 1.2 pH with hydrochloric acid. Both samples were heated in a water bath for 5 h at 180°F. The chromatogram for the levulose sample prior to heating, run by the straight silylation

[7] Meade, George P. 1964. Spencer-Meade Cane Sugar Handbook. Ed. 9 p. 459. Wiley Co., N. Y.

TABLE 10--Dextrose-levulose content of invert solutions prepared with invertase

Samples	% Of total sugar		% In solution	
	% Levulose	% Dextrose	% Levulose	% Dextrose
5% Sucrose solution	48.8	51.2	2.4	2.6
10% Sucrose solution	49.7	50.3	5.0	5.1
50% Sucrose solution	49.8	50.2	26.8	26.9

GLC method, without oxime formation, outlined in the experimental section is shown in figure 2. After heating at pH 1.2 for 5 h, three major new peaks - 6, 7, and 8 - were found (figure 3). The earlier peaks (retention time of 2 to 7 min) are attributed to levulose isomers. The areas were calculated for the three new major peaks, and their concentration estimated by determining their percentage of the total area of all the peaks.

$$\frac{\text{Area of peak}}{\text{Total area of all sugar peaks}} \times 100 = \% \text{ of Total peak area}$$

Since the GLC response factors for these compounds are not known, nor is their precise identity, this means of calculating concentration is only valid for estimating concentration changes. Table 11 is a tabulation of the changes occurring for peaks 6, 7, and 8. It should be noted that the major constituent formed, peak 7, has a retention time similar to sucrose, and that very little of this compound was formed at 4.0 pH.

TABLE 11--Changes in chromatogram of levulose upon heating

Samples	Hours at 180°F	% Of total peak area		
		Peak 6	Peak 7	Peak 8
50% Levulose solution at 4.2 pH	0	0	0	0
	1	0	0	0
	5	0	1.5	0
50% Levulose solution at 1.2 pH	1	0	2.2	1.6
	3	1.1	4.8	2.8
	5	2.1	8.2	2.4

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	1	0	0	0
	5	0	1.5	0
50% Levulose solution at 1.2 pH	1	0	2.2	1.6
	3	1.1	4.8	2.8
	5	2.1	8.2	2.4

Individual samples of levulose, dextrose, and sucrose were prepared and subjected to the same pH and temperature conditions as in the previous experiment. Chromatograms were run by the straight silylation procedure. Again the levulose solution produced almost identical concentrations of peaks 6, 7, and 8 under acid conditions (table 12). The dextrose solution on acid treatment only produced trace amounts of peaks 9 and 10 (as shown in figure 4 and table 12).

TABLE 12--Changes in chromatograms of levulose, dextrose, and sucrose upon heating

Samples	Hours at 180°F	% Of total peak area				
		Peak 6	Peak 7	Peak 8	Peak 9	Peak 10
60% Lev. soln. at 4.0 pH	0	0	0	0	0	0
	5	0	1.0	0.4	0	0
60% Lev. soln. at 1.5 pH	1	0	2.4	2.6	0	0
	3	0.9	5.7	2.0	0	0
	5	1.5	6.9	2.9	0	0
60% Dex. soln. at 5.2 pH	0	0	0	0	0	0
	5	0	0	0	0	0
60% Dex. soln. at 1.6 pH	3	0	0	0	0	0
	5	0	0	0	Trace	Trace
60% Suc. soln. at 5.8 pH	0		100[1]	0	0	0
	5		100[1]	0	0	0
60% Suc. soln. at 1.6 pH	1	0	0.6	0.8	0.6	0.2
	3	0.3	1.7	0.3	0.4	0.1
	5	0.7	3.0	0.3	0.6	0.3

[1]Sucrose is being measured in these samples since sucrose has same retention time as peak 7.

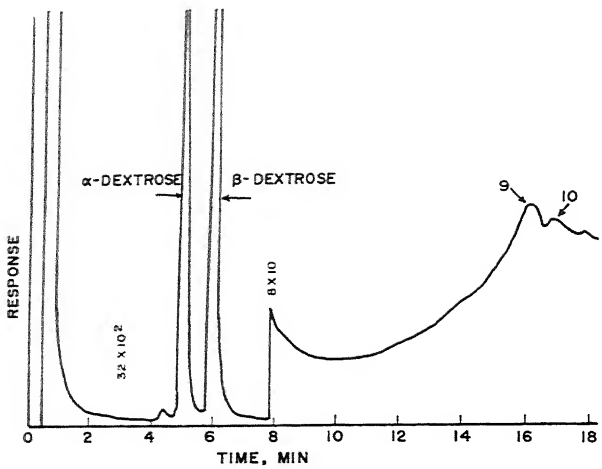

FIGURE 4--Dextrose solution, pH 1.6, heated 5 h at 180°F.

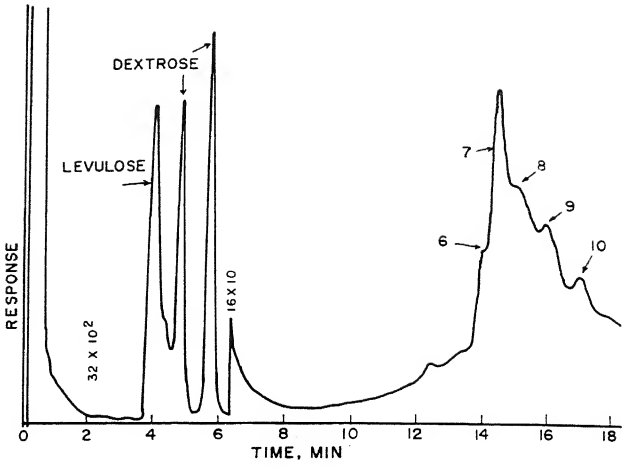

FIGURE 5--Sucrose solution, pH 1.6, heated 5 h at 180°F.

The sucrose solution on heating at low pH revealed all five peaks, as would be expected if the sucrose was inverted totally to dextrose and levulose (figure 5 and table 12). Again, peak 7 was produced in the greatest quantity. It was, therefore, apparent that the higher sugars are being formed mainly from levulose while dextrose is fairly stable under these conditions.

IDENTIFICATION OF HIGHER SUGARS PEAKS

It has been reported that levulose under acid conditions can form difructose dianhydrides[8], and we were able to obtain samples of two of these dianhydrides from Dr. W. W. Binkley[9] along with their chemical structures (figure 6). Our GLC analyses of the silyl derivatives indicated that difructose dianhydride I has the same retention time as peak 6, and difructose dianhydride II has the retention time of peak 7 (figure 7). The retention times of the two dianhydrides also agreed with the retention times for 6 and 7 peaks when the temperature was programmed at 2°/min rather than 6°/min.

When difructose dianhydride I was added to a sample of levulose, which had been heated at low pH for 5 h, peak 6 increased; on the addition of difructose dianhydride II, peak 7 increased with the chromatograms showing good clean peaks. This confirmed that their retention times are identical, and, based on the fact that these dianhydrides have been known to be formed under acid conditions, there is certainly a very good possibility that peaks 6 and 7 represent difructose dianhydrides I and II.

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[8]Wolfrom, M. L., and Blair, M. G. 1948. Action of heat on D-fructose. Isolation of diheterolevulosan and a new di-D-fructose dianhydride. J. Am. Chem. Soc. 70: 2406-2409.

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60% Lev. soln. at 1.5 pH	1	0	2.4	2.6	0	0
	3	0.9	5.7	2.0	0	0
	5	1.5	6.9	2.9	0	0
60% Dex. soln. at 5.2 pH	0	0	0	0	0	0
	5	0	0	0	0	0
60% Dex. soln. at 1.6 pH	3	0	0	0	0	0
	5	0	0	0	Trace	Trace
60% Suc. soln. at 5.8 pH	0		100[1]	0	0	0
	5		100[1]	0	0	0
60% Suc. soln. at 1.6 pH	1	0	0.6	0.8	0.6	0.2
	3	0.3	1.7	0.3	0.4	0.1
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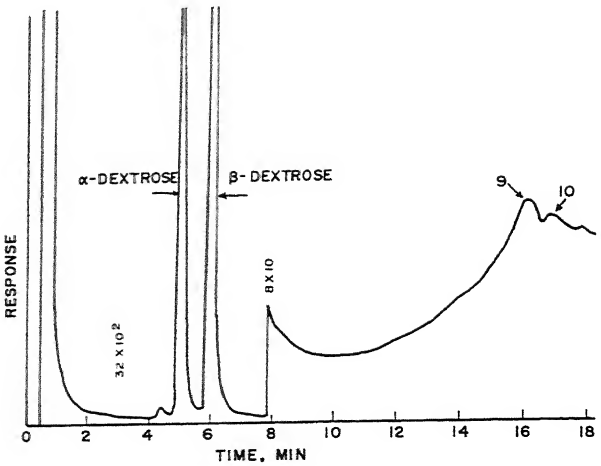
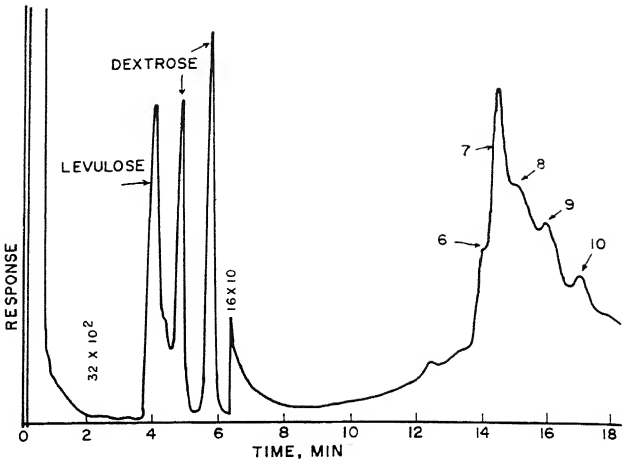


FIGURE 4--Dextrose solution, pH 1.6, heated 5 h at 180°F.



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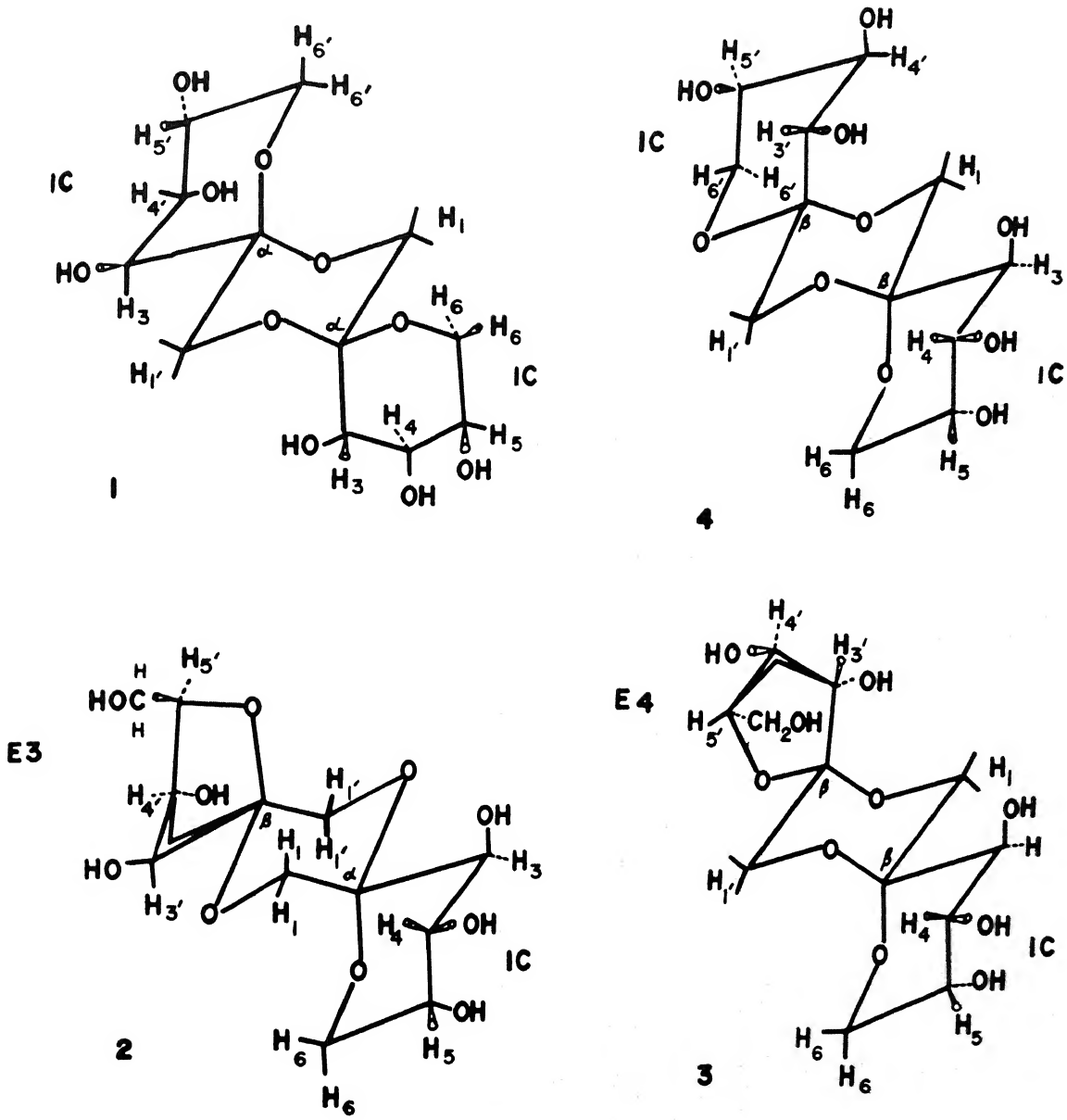


FIGURE 6--Difructose dianhydrides

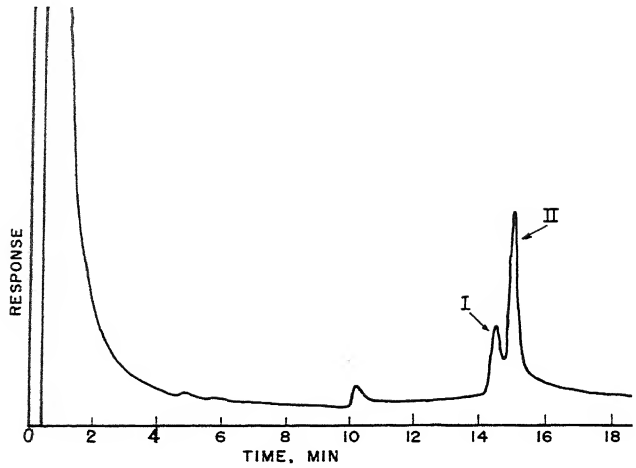
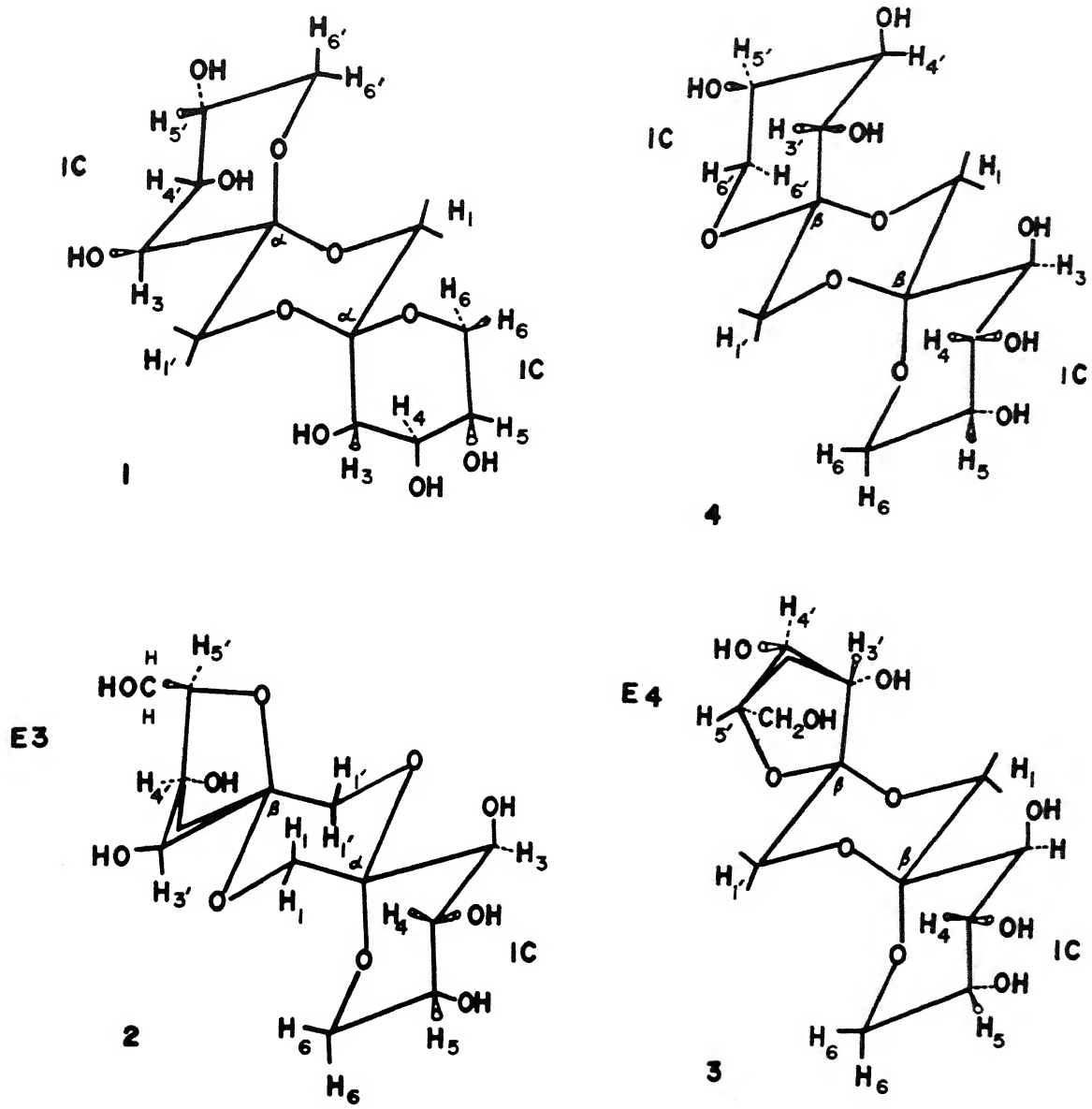


FIGURE 7--Difructose dianhydrides I and II

TABLE 13--R_f values for sugars, invert and difructose dianhydrides

Spot No.	Spot color	Heated lev. pH 1.0	Heated dex. lev. pH 1.0	Heated suc. pH 1.0	Lev.	Dex.	Suc.	Difr. dian. I	Difr. dian. II
1	gray	0.18	0.18	0.18	-	-	-	-	-
2	gray	0.25	0.25	0.25	-	-	-	0.25	-
3	gray	0.31	0.31	0.31	-	-	0.31	-	0.31
4	yellow	0.40	0.40	0.40	0.40	-	-	-	-
5	blue		0.46	0.46	-	0.46	-	-	-

The GLC results were confirmed by thin-layer chromatography. The solvent mixture used was n-butanol, acetic acid, ether, and water (50:30:15:5). The samples were spotted on silica gel G adsorbent treated with 0.1N boric acid. The results (table 13) showed that the R_f of both difructose dianhydride I and spot number 2 of the test solutions was 0.25. In addition, the R_f of difructose dianhydride II, sucrose, and spot number 3 was 0.31. Further verification analyses are generally required to prove definitely the identification of these peaks.



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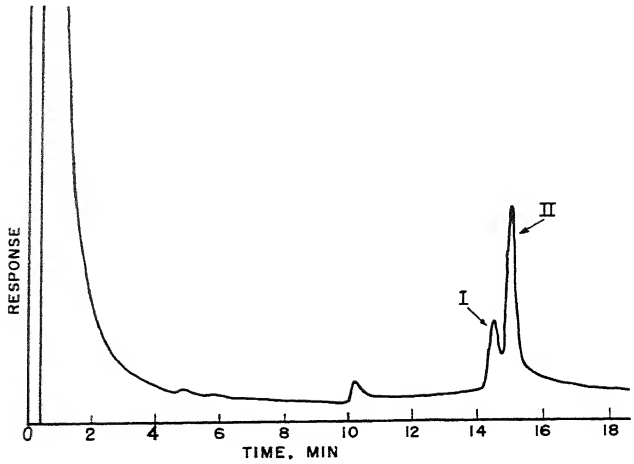

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3	gray	0.31	0.31	0.31	-	-	0.31	-	0.31
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CONCLUSIONS

Although this initial investigation has created many questions about the stability of levulose during the manufacture of invert sirups, the following conclusions can be made:

1. There is less levulose than dextrose in most manufactured invert sirups.

2. Levulose appears more sensitive to low pH/high temperature conditions than dextrose.

3. Levulose forms higher sugars under acid conditions; two of the compounds formed could be difructose dianhydrides I and II.

4. Sucrose inverted with invertase appears to undergo less destruction of levulose than that subjected to acid methods. Therefore, invertase would be recommended as an inverting agent in analytical methods.

Appendix--Procedure for sugar analysis

Special apparatus:

1. Gas Chromatograph: F&M Model 810 or equivalent equipped with flame ionization detector and Hewlett Packard Model #3373-B Integrator.

2. Stainless steel column, 1/4-inch by 6-feet, packed with 5% silicon rubber SE-52 coated on 80/100 mesh chromosorb G-A.W. DMCS. The column is available ready-packed from Hewlett Packard.

3. Syringe: Hamilton No. 701 NW/G, 10 µl capacity.

4. Oil bath maintained at 75°C.

Reagents:

1. Hydroxylamine hydrochloride: Baker Analyzed Reagent from J.T. Baker Chemical Co., Phillipsburg, N. J.

2. Pyridine: certified A.C.S. from Fisher Scientific Co., Springfield, N. J.

3. Hexamethyldisilazane: specially purified grade from Pierce Chemical Co., P. O. Box 117, Rockford, Illinois.

4. Trifluoroacetic acid reagent: 99+% pure from Pierce Chemical Co.

5. Phenyl beta-D-glucoside from Nutritional Biochemical Corp., Cleveland, Ohio.

6. D-Fructose from J.T. Baker Chemical Co.

7. Dextrose from National Bureau of Standards, Washington, D. C.

GLC conditions:

1. Column oven temperature: Initially at 170°C - raise temperature at 4°C per minute to 300°C.

2. Detector temperature: 350°C

3. Injection temperature: 300°C

4. Gas Flow Rates:

Carrier gas (Helium)	- 40 ml/min
Hydrogen	- 60 ml/min
Air	-600 ml/min

5. Attenuation: Settings that would provide peaks having 1000 to 1000 x 10^3 counts.

Preparation of TMS sugar-oximes

1. Weigh accurately 400-500 mg sugar sample (60° Brix or over). In very dilute samples water can be removed by using a rotary flash evaporator under vacuum and 70°C temperature bath.

2. Using pyridine transfer the samples to a 100 ml volumetric flask containing accurately weighed phenyl-beta-glucoside (200 mg), and make up to volume.

3. Place 2 ml aliquot to a 50-ml volumetric flask containing 25 mg hydroxylamine hydrochloride.

4. Stopper the flask loosely and submerge in a 70°C oil bath using clamps and iron stand.

5. Continue heating for 30 minutes with occasional swirling.

6. Remove from the bath, and add 5 ml hexamethyldisilazane and 0.5 ml trifluoroacetic acid.

7. Mix, stopper, and set aside for 30 minutes.

8. Inject 5 µl into the GC.

DISCUSSION

R. A. KITCHEN (B.C. Sugar): After your oximation reaction, did your GLC work ever indicate more than one peak for a specific sugar?

V. S. VELASCO: No; as long as there is an excess amount of hydroxylamine hydrochloride, there is only one peak. I have never seen more than one peak using this method on levulose and dextrose.

R. A. KITCHEN: Have you tried more than one column packing?

V. S. VELASCO: We used only 5% silicone rubber on Chromosorb G; that is, the SE52 column.

R. A. KITCHEN: Depending on the conditions of the oximation reaction, you may not get only an acyclic oxime derivative formed. You can get at least two cyclic compounds formed as well; that is, substituted hydroxylamine derivatives with the sugar in either the α- or the β-anomeric form.

V. S. VELASCO: We did not find any of these minor peaks, but we were running the instrument at a relatively high sensitivity, at a setting of 1 attenuation and a 10^2 range, when we obtained this single peak. Perhaps if we were to make a run using a much higher sensitivity, we would see these additional peaks.

R. A. KITCHEN: In reactions of unsubstituted sugars with phenylhydrazine, thiosemicarbazide and tosylhydrazine, I have found that the crystalline products contained high concentrations of the cyclic derivatives. I determined the concentration of the acyclic form by detection of the peak due to the methine proton using NMR spectroscopy. In one oximation reaction, I have also isolated a partially crystalline product which was shown to be a hydrated form of the oxime. M. L. Wolfrom and other workers[1-8] reported several cyclic derivatives, as well as some of the difructose dianhydride compounds.

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[1]Wolfrom, M. L. and Blair, M. G. 1948. Action of heat on D-fructose. Isolation of diheterolevulosan and a new di-D-fructose dianhydride. J. Am. Chem. Soc. 70: 2406-2409.

[2]Wolfrom, M. L., Binkley, W. W., Shilling, W. L., and Hilton, H. W. 1951. Action of heat on D-fructose. II. Structure of diheterolevulosan II. J. Am. Chem. Soc. 73: 3553-3557.

[3]Wolfrom, M. L., Hilton, H. W., and Binkley, W. W. 1952. A new di-D-fructose dianhydride. J. Am. Chem. Soc. 74: 2867-2870.

[4]Shamgar, A. H. and Leibowitz, J. 1961. Autotransformation of D-fructose. J. Org. Chem. 26: 285.

[5]Lemieux, R. U. and Nagarajan, R. 1964. The configuration and conformation of "di-D-fructose anhydride I." Can. J. Chem. 42: 1270-1278.

[6]Wolfrom, M. L. and Christman, C. C. 1931. The occurrence of true hydrazone structures in the sugar series. J. Am. Chem. Soc. 53: 3413-3419.

[7]Wolfrom, M. L., Fraenkel, D. R. Lineback, F. C., and Komitsky, J. M. 1964. Structural investigation of acetylated sugar phenylhydrazine derivatives. J. Org. Chem. 29: 457-461.

[8]Holker, J. R. 1964. Sugar thiosemicarbasones. Chem. Ind. 13: 546.

M. A. GODSHALL (C.S.R.R.P.): How did you eliminate the water from your solutions before you prepared them for GLC?

V. S. VELASCO: We always used about 50% sugar solutions. We found that it is not necessary to eliminate the water as long as you use excess silylating reagent. However, with the standard invert solution, which is only 5% to 10% solids, we did evaporate the water in a rotary evaporator, under vacuum, at 70°C.

M. MATIC (Sugar Milling Research Inst.): To clarify the point: do you find three isomers or only one after silylation of the oximes?

J. F. DOWLING (CPC International): We see only one peak; however, the situation could be as Dr. Kitchen describes, if peaks from the other isomers were hidden under the one main peak. With straight silylation, of course, without preparing the oxime, there are two peaks.

M. MATIC: That is how I had heretofore understood this situation, but now this question of three oxime peaks has been raised. When you compared the results from your two methods--with and without the oxime--the results were exactly the same, if I recall correctly. If there are additional oxime isomers, then additional peaks should show, unless as you said they are under the one peak that you found, and that one peak looked very symmetrical to me. I am especially interested, because we had been thinking of trying the oxime method.

R. A. KITCHEN: Do you think that your oxime method is better than other methods, for example, the use of sodium borohydride followed by silylation?

V. S. VELASCO: Yes, I think so, because it eliminates fluctuations in the GLC data, and the necessity for making corrections in the quantization of dextrose.

E. J. ROBERTS (C.S.R.R.P.): We have tried the commercial oxime-forming reagent, "STOX,"[9] in the GLC analysis of sugars. We found that the trimethylsilylated oxime of fructose gave only one peak, but those of glucose and several other sugars gave two peaks. This was not surprising, since the sugar oximes mutarotate and may also exist in the syn- and anti- forms. The results obtained with the oximes may depend upon the resolution of the chromatographic column used, and upon operating conditions.

V. S. VELASCO: "STOX" is the reagent that includes both the internal standard and the oxime-forming reagent. After the oxime is formed, the silylation is done in the usual fashion.

[9]Pierce Chemical Company, Box 117, Rockford, Illinois 61105.

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